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METHODS for EVALUATION of NUTRITIONAL ADEQUACY and STATUS

—a *Symposium*

ADVISORY BOARD on QUARTERMASTER
RESEARCH and DEVELOPMENT

Committee on Foods
December 1954

DEPARTMENT OF THE ARMY
OFFICE OF THE QUARTERMASTER GENERAL

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METHODS FOR EVALUATION OF NUTRITIONAL ADEQUACY AND STATUS

A symposium sponsored by the
QUARTERMASTER FOOD AND CONTAINER INSTITUTE
For the Armed Forces
QUARTERMASTER RESEARCH AND DEVELOPMENT COMMAND
U. S. ARMY QUARTERMASTER CORPS
and
MEDICAL NUTRITION LABORATORY
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December 1954

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Preface

Recognizing its responsibility to maintain good nutrition in the soldier as well as its responsibility to civilian population groups in occupied countries and to military assistance groups, the Army has over a period of years been interested in methods for evaluating the nutritional adequacy of foods and the nutritional status of the people who consume them. While we may be justly proud of our achievements in military feeding we are frequently frustrated in our attempts to obtain a rapid and meaingful measure of the nutritional adequacy of our feeding program.

The initial approach to the measurement of nutritional status was teleological. Today we are aware of "nutritional status" as an ecological concept. The response of the individual to his external and internal environments determines his metabolic demands and these in turn must be translated into nutritional requirements. Body size, and its component compartments as well as surface area; work output; climate; sex; and age are all factors determining calorie expenditure. These in turn can be modified by the nutriture, as for example, the decreasing calorie requirements of starving people. Protein requirements cannot be fulfilled until calorie demands are satisfied. The vitamins and other accessory food substances must be present in proportion to the calories and quality of protein. Such understandings are needed now more than ever to evaluate our strength and weakness in military preparedness in the cold war which engulfs us today on a global scale. The symposium on Methods for Evaluation of Nutritional Adequacy and Status was arranged under the joint sponsorship of the Nutrition Division of the Quartermaster Food and Container Institute for the Armed Forces and the Medical Nutrition Laboratory, Office of the Surgeon General to bring together the most authoritative and most recent information in this field of mutual interest.

The objectives of the symposium were admirably accomplished:

(1) The merits of existing methods were critically evaluated,

(2) The needs for special methods not now available were emphasized, and

(3) The possibilities for developing such methods in the near future were constructively considered by appropriate suggestions.

The importance of the subjects discussed to a broad spectrum of technical specialists as well as to the responsible personnel of the sponsoring technical services is indicated by the wide range of scientific disciplines, academic and government agencies and geographic areas represented by the participants and those in attendance.

A better appreciation of the complexity of the relationships of food composition to body composition and function was achieved by the diversity of subjects upon which attention was focused. Far from being discouraging this appreciation tends to emphasize the benefits to be derived from fundamental research and to underline the promise offered in such basic approaches as metabolic studies in microorganisms and measurement of enzyme activity. The importance of using several species of experimental animals was brought out in the discussion of animal techniques for determining the physiological adequacy of the nutrients in the diet. Evaluation of the nutritional status of individuals or surveys of population groups is now more confidently made by combining clinical examination with biochemical assessment. The potential offered by the embryonic field of measurement of body compartments as an index of nutriture was enthusiastically reported in the round table discussion.

The sponsoring technical services now have a broader basis for interpreting the significance of the various measures of nutritional adequacy and status and are encouraged by the profitable lines of future investigation suggested.

One would be remiss indeed not to express appreciation for the good planning and the effective management that made the symposium so successful. For these contributions the sponsoring groups deserve high commendation. The appreciation of all who participated is also due to Colonel John D. Peterman, commandant of the Quartermaster Food and Container Institute, whose address of welcome established the cordial and friendly atmosphere of the meetings, and to Lieutenant Colonel Robert Ryer, III, who assisted in innumerable ways in coordinating the program for the Medical Nutrition Laboratory.

HERBERT POLLACK, M. D.

Consultant on Nutrition to The Quartermaster General and The Surgeon General, U. S. Army

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Proceedings of the Symposium

METHODS FOR EVALUATION OF NUTRITIONAL ADEQUACY AND STATUS

I. Introduction

THURSDAY MORNING SESSION

25 February 1954

DR. ELMER N. NELSON, *Presiding*

CHAIRMAN NELSON:

In calling the symposium to order, I wish first of all to call your attention to the overall organization of the program. Today, you will note, we begin with a statement of our objectives and then proceed to a consideration of various methods of evaluation—methods of evaluating protein adequacy, vitamin adequacy, and mineral adequacy. Tomorrow, we discuss animal experimentation, the nutritional status of populations, and conclude with a roundtable on body composition. Into this framework it should be possible to fit most of the things that are of concern to nutritionists.

Our first paper, which will keynote the symposium, is by Dr. Charles Glen King, scientific director of the Nutrition Foundation, Inc.

The Problems, Purposes, and Scope of the Symposium

CHARLES GLEN KING

Scientific Director, Nutrition Foundation, Inc., and Professor of Chemistry, Columbia University

We hear much of the mysteries of the atom these days. All of us have at least a vague concept of the complexity of research in atomic physics and in radiation chemistry, but unless we are working in that field, we take much for granted. The mysteries of the atom, however, may be of less significance and relatively simple, compared with the mysteries associated with the functioning of the human body.

As scientists, our approach to the latter field is necessarily in terms of atoms as a starting point. But the problems of function and organization, including such concepts as optimum health through a life span, and the highest levels of performance under the tensions and stresses of military and civilian life, take us into an area where we risk underestimating the complexity of our problems.

If modern biological research has taught us anything fundamental, it is to instill an appreciation of the complexity and sensitivity of our body structures and the relationships of these changes to our performance as human personalities. Unless we have this viewpoint in our approach to the problems of military feeding, I think we fall far short of meeting our responsibilities.

For example, almost every year through the last three decades, scientists have discovered new essential nutrients. The number of newly discovered nutrients that are useful, though not essential, is much greater. To identify the nutrients and to learn how to measure them by chemical and physical procedures, represents a field in which progress is rapid and of great value. But beyond this kind of basic information is the more difficult field of study—to discover how the nutrients function. Then we are faced with the practical problems of nutrition, to keep each individual at or near his optimum performance. Our estimate of performance should include the momentary reactions and morale of an individual under conditions of the most severe physiological and mental stress, in addition to maintaining an overall sane outlook in terms of life span and a sound economy in our total social structure.

In our discussions at this conference, I would like to emphasize the fact that we face great risks at the two extremes of our discussions. First, perhaps the greatest risk (as observed over and over during World War I and World War II) is that we will underestimate the value of our targets as we move ahead, even though we superficially attempt to use the full flow of new research findings. And second, while many of us keep our main attention upon the research frontier, others are under a stern discipline to be practical—they must act in terms of our national economy and our total resources for defense and offense through an indefinite period—they are scarcely acquainted with either the significance of research or the people who do research.

To illustrate the first point, during every prolonged military campaign in history, so far as I can find, the military forces—and usually the civilian population as well—had to contend with massive outbreaks of nutritional deficiency diseases. According to one of the most competent nutrition scientists in Russia, almost the whole Russian Army was in a moderate to well-established state of scurvy at the time of their collapse in World War I. Although there were relatively few outbreaks of clinical deficiency disease among the armed forces of the major contending nations in World War II, there were reliable reports of folic acid deficiencies among troops in India, and the prison camp feeding conditions in both Europe and Asia were frequently shocking. Feeding our aviation forces to accomplish their best performance was only slowly and perhaps never satisfactorily accomplished. We can note with some satisfaction, however, the substantial progress that has been made in developing more efficient rations during and since the active stages of World War II.

I well recall the overconfidence that all of the nutrients of practical significance were in the rations when field tests were being planned and conducted at Fort Lee. Some of us urged that a control group be provided to furnish more adequately the unidentified nutrients that might be present in such foods as milk, eggs, fresh leafy vegetables, and liver, but plans for the experiment had gone too far to include such a group when the matter was first brought to the attention of the responsible personnel. Some of you may recall, too, the long and repeated arguments that were required before we could get provision for regular biological testing of the rations. The strong and entrenched tradition was to rely only upon the field performance of men, at great expense of time, money, and urgently needed manpower. And may I emphasize that possibly the most important consideration in this kind of situation is the risk of less than optimum performance of manpower during the interval of getting the right answer.

Not in a spirit of criticism, but simply as a factual guide to our future course, we should note that some of the packaged rations in use during World War II were inadequate according to standards that most of us would accept at the present time. Among the principles of nutrition then not known to have significance in human feeding, we might cite such items as the role of folic acid and vitamin B_{12}, and the critical importance of balances between amino acids and members of the vitamin-B complex, especially as they pertain to vitamin B_6; the protective effect of a generous carbohydrate intake at high altitudes; the value of high protein intakes under other stress conditions; the value of saline water and other special nutrients during convalescence from shock, hemorrhage, burns, and infections—and numerous other areas where there has been notable progress since 1945.

May I congratulate the officers and their advisers for their insight and diligence in setting up a symposium such as you have organized for today and tomorrow. The emphasis here upon advances in basic information, and their implications for usefulness on behalf of the Armed Forces and civilians, is an occasion for feeling grateful.

Importance of Nutritional Status and Methodology to the Army

LT. COL. TYRON E. HUBER

Research and Development Division, Office of The Surgeon General

There is hardly anyone who would object to the thought that nutrition has been of tremendous importance from time immemorial in the waging of combat. It has required much effort of many individuals to secure adequate and proper nutrition of soldiers, and this effort continues to engage our resources and research facilities in solving or in the attempt to solve some of the problems that confront us.

Gross nutritional deficiencies seldom exist in the Army of today. When they do exist, generally they are diagnosed quickly and adequately treated. However, there are some minor deficiencies which, over a long period of time, conceivably lead to a reduction of stamina and physical fitness of combat personnel, to increased delay in wound healing, and, in certain instances, to lessened resistance to disease.

The Surgeon General of the Army is interested in all these aspects, particularly in the last two; namely, the relation of nutritional status to resistance to disease and to wound healing.

The Surgeon General of the Army is charged with the responsibility of maintaining the physical fitness of combat and military personnel of the Army. By Army Regulations he is charged with the responsibility of prescribing basic diets for all field conditions. In addition, he is charged with the responsibility of making recommendatons regarding food and the feeding of troops and military personnel.

In the field, Medical Service officers are responsible for providing a nutritive diet to the troops and, under certain circumstances, for the methods used in determining that the troops receive a nutritive diet. These responsibilities are clearly defined by Army Regulations.

We know the fields for which we are responsible. What is not clear is the manner in which to fulfill those responsibilities. Fortunately, we are allowed a considerable degree of latitude in employing methods for the accomplishment of the requirements for which we are responsible. However, there are a number of points on which there is divergence of opinion as to the best methods, techniques, and procedures to be employed in determining the adequacy of nutrition.

I am sure there will be several speakers today who will point out those differences of opinion and it is our hope that the Army Medical Service will provide a small contribution in this field and, at the same time, obtain information that may be utilized in carrying out our responsibilities.

You may well ask what the Army Medical Service is accomplishing in its attempt to improve techniques, methods, and procedures. A number of research studies at the Medical Nutrition Laboratory, Fitzsimons Army Hospital, Denver, Colo.; the Army Medical Research Laboratory, Fort Knox, Ky.; and the Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C., are presently retesting these techniques in various nutrition investigations. A portion of the results of these investigations will be presented during this symposium.

It is, indeed, gratifying that we were invited to come as guests and to participate in this symposium, that we may receive from you certain thoughts concerning procedures and techniques. The most precious thing, by far, that we can attain today is ideas; and with knowledge and ideas, we hope to make progress. We believe that only through a free exchange of ideas can progress be made and can we fulfill our responsibilities.

Controls in Measuring Nutritional Response

E. W. Crampton

Department of Nutrition, MacDonald College (McGill University)
Province of Quebec, Canada

In biological research a control is a benchmark, a measurement to furnish a datum level—or perhaps a touch-stone by which to judge related criteria. The control is the basis for the interpretation of the investigation. A faulty control may contribute to false conclusions, and the absence or loss of the control unit jeopardize an entire study. It is not surprising, therefore, that the research worker is acutely conscious of the integrity of his controls. His problem is not made simpler by the fact that with biological criteria the control, quantitatively, is specific for each study and hence must be defined in each case. Furthermore, it frequently happens that the criteria may be considerably influenced by numerous environmental and/or hereditary factors which becloud the clear and accurate description necessary to establish whether or not comparative observations really differ. This situation is particularly characteristic of data which must be collected by methods typical of surveys, as distinct from experiments in which imposed experimental conditions can be arranged. Furthermore, the desirability of considering individuals as distinct from groups introduces special problems.

Inasmuch as the description of criteria used in nutritional studies is largely numerical, the research worker usually finds that statistical methods are necessary to clarify and precisely describe the data, and subsequently to establish whether or not intentionally imposed or naturally occurring conditions have really effected a change in the criteria under consideration.

I am much aware of the fact that statistics is not in the best of repute among some biological workers. A considerable number may see something beside humour in the recent notice of a new book entitled *How to Lie—(With Statistics)*. In defense of the statistical approach, however, I suggest that when properly used, statistics becomes a rather special tool to assist in the elucidation of data which may otherwise be so interrelated as to conceal some of its important facts. Our experience has been that statistics carelessly or wrongly used usually proves to be anything but helpful, though if properly applied may accomplish results beyond the possibilities of other methods.

Our laboratory has been for many years and still continues to be interested in the application of statistical methods to nutritional research data. In 1938, for example, we published a description of the use of a combination of the analysis of variance and partial regression in the reduction and interpretation of data obtained from studies of factors related to, and presumably partly responsible for, the extent of the development of the musculature of the bacon pig. The criteria used, however, were different from those applicable to human nutritional or health data, and I shall not refer to them directly in this paper.

On the premise that it will facilitate presentation to employ—in preference to a series of appropriate mathematical formulae—a worked-out example, I have prepared and analysed a set of hypothetical data.

The data are intended to represent the results of examinations of 6 groups of 50 male subjects each. The studies are to be considered as having been carried out during each of 2 years at 3 stations—1 in an arctic area, 1 in the temperate zone, and 1 in the tropics. The criterion of nutritional status or of physical fitness is: the time in seconds required for the heart rate to return to its previous resting rate, following an exercise tolerance test. This criterion is referred to as the Heart Recovery Time. It is the dependent variable in our analysis and is indicated in formulae by the letter y. Four other items were also recorded for each of the 300 subjects, viz: x_1, age in years; x_2, weight in kilograms; x_3, skinfold thickness in centimeters; and x_4, calories intake per day.

These variables are of two distinct types. Those of time and place are discreet in this example. The others are continuous; that is, all intermediate values between the extremes may occur. Each, however, may have affected the dependent variable.

The first step in the reduction of the data is the calculation of the averages of each variable. These values, however, will often not be too useful in dealing with the individual subject. Truly there is no average man. What we seek as the benchmark or the control is the *expected* Heart Recovery Time applicable to a given individual as we find him—in the Tropics or the Arctic, this year or some other year, and of his own unique age, weight, fatness, and appetite.

To obtain this *expected* figure we require to know the variability between individuals, uninfluenced by time and place, but with full account of the individual attributes (with respect to the four independent variables). We can obtain such a value by the use of a combination of two statistical devices. First, an analysis of variance for the discontinuous variates will establish the variability between subjects comparable as to year and station; and, eventually, in combination with the coefficient of multiple correlation give us a measure of the reliability of our control figure. Secondly, the application of multiple correlation and partial regression will enable us to arrive at an *expected value* for the dependent variable in accordance with the relationships which exist between the four continuous variables.

The essential description of the "raw" data is shown in table 1.

TABLE 1

MEAN VALUES AND STANDARD DEVIATIONS OF VARIATES

Variate	Means	Standard deviations	
		Units	Percent (approximate)
y, Heart recovery time (all groups)		±76	
1st year survey	95 sec		
2d year survey	85 sec		
Arctic area	80 sec		
Temperate area	90 sec		
Tropical area	100 sec		
Mean of dependent variable (second)	90 sec	±20	22
x_1, Age (years)	30 yr	±5	17
x_2, Weight (kilograms)	70 kg	±10	15
x_3, Skinfold (centimeters)	3 cm	±1	33
x_4, Calories	3,500 cal	±280	8

The standard deviations of table 1 are obtained from an analysis of variance of the data for the dependent and for each of the independent variates. An example of the appropriate analysis for the dependent variate y (Heart Recovery Time) is shown in table 2.

TABLE 2

ANALYSIS OF VARIANCE OF HEART RECOVERY TIME, y

Source	Degrees of freedom (D/F)	$S(x-x)^{-2}$	Variance s^2	Standard deviation s	Freedom ratios	
					Observed	Necessary (P.05)
All causes	299		5, 800	±76		
Subgroupings	5		5, 400			
Between years	1		600		1. 5	3. 86
Between stations	2		4, 000		10. 0	3. 02
Interaction	2		800		2. 0	3. 02
Between individuals in the same year at same station (error).	294	117, 600	400	±20		
Accounted for by multiple correlation (=R².117,600).	4	62, 657				
Standard error of estimate (adjusted standard deviation).	290	54, 943	190	±14		

Included in this table are three entries under the heading Sums of Squares, $S(x-x)^{-2}$. This heading is not usually shown because it can be readily found by multiplying the variance by its corresponding "degrees of freedom." It is included here only to illustrate the use of the multiple correlation coefficient in obtaining the "error of estimate" applicable to the expected value to be calculated from the regression equation.

Table 2 indicates that the difference in the average Heart Recovery Time between the 2 years is not a real effect of time. There are, however, significant differences to be expected between the three geographic areas. There is no interaction between years and stations. We can generalize, then, to the extent of being reasonably assured that the mean numerical value of our criterion will be valid for either year and will not be differentially modified by the geographic area in which observations are made. In comparing individuals from different stations, however, some correction from the general mean of all groups must be applied. Corresponding analyses would give a basis for the same type of interpretation for each of the other continuous variates.

It now becomes necessary to calculate from the "error" line of their respective variance analyses the simple correlation coefficients (r) between each of the continuous variables. Once these 10 values are at hand they are inserted in appropriate positions in a set of normal equations, the subsequent solving of which yields 4 beta (β) values. These latter, together with the correlation coefficients, permit calculation of the multiple correlation coefficient (R). One further step remains—the calculation of the partial regression coefficients (b_{yx}) by solving an appropriate equation for each of the four (x) variates. Once these several statistics have been obtained they may be assembled as in table 3.

TABLE 3

BETA VALUES, PARTIAL REGRESSION COEFFICIENTS, AND MULTIPLE CORRELATION COEFFICIENT

Independent variate	Betas β	Relative weight of β in percent	Partial regression coefficients
x_1, Age (yr.)	−0.2470	10	−1.1
x_2, Weight (kg.)	.3736	14	.75
x_3, Skinfold (cm.)	1.2457	46	24.9
x_4, Calories	−.8129	30	−0.058

Multiple correlation: $R^2=0.5328$ and $R=0.73$.
Necessary R for P 0.05=0.16.

We may now consider what these statistics tell us. First we note the multiple correlation coefficient is statistically significant; and next that 53.28 percent of the variability in Heart Recovery Time between men of the same locality is accounted for by the four independent variables considered in the multiple correlation. Obviously there are still unaccounted for factors affecting this criterion, though as we shall see, the "expected" value of (y) determined from the four independent variables is an appreciably more accurate "norm" than is the average of any group.

We learn from the betas that of the total influence of all four of the independent variables on Heart Recovery Time, 46 percent of it is traceable to the degree of fatness of the subject, and 30 percent to the amount of food eaten daily. Age and weight have relatively less effect.

The partial regressions tell us (within the range of the observed data) how much the Heart Recovery Time can be expected to be changed from the general average, for every unit that any one of the factors of age, weight, fatness, and calorie intake of the individual subject differs from the average of these factors found in the 300 subjects examined. Thus, for each 1 year of increase in age from the average age of 30 years, Heart Recovery Time will be expected to decrease 1.1 seconds when the other 3 variables are constant; but for each centimeter increase in skinfold thickness over the average of 3 cm. the Recovery Time will be delayed about 25 seconds.

We may now assemble the partial regressions and the averages of the continuous variates into an equation from which to calculate for individuals according to their own "measurements" an *expected* Heart Recovery Time (Y).

$$\begin{aligned} Y &= \bar{y} + byx_1(x_1-\bar{x}_1) + byx_2(x_2-\bar{x}_2) + byx_3(x_3-\bar{x}_3) + byx_4(x_4-\bar{x}_4) \\ &= 90 - 1.1(x_1-30) + 0.75(x_2-70) + 24.9(x_3-3) - 0.058(x_4-3500) \end{aligned}$$

At this point I wish to refer again to table 1 where it is shown that the standard deviation of Heart Recovery Time in the "raw" data was ± 76 seconds. When the variability due to the discontinuous variables of time and station is accounted for, this standard deviation is reduced to ± 20 seconds. If account is now also taken of the 4 independent variables, the standard deviation of our "Expected Recovery Time" becomes ±14 seconds. This is a coefficient of variation of approximately 15 percent.

We may now calculate for a 35-year-old man at a tropical station, weighing 70 kg. and showing 4.5 cm. skinfold thickness, and a 3,800 calorie intake, $Y=106$ seconds, to which we must add 10 seconds to adjust for the fact that the "normal" Recovery Time for the tropical station is 10 seconds longer than the general mean. Our expected value, then, for this man becomes:

$$Y = 116 \text{ seconds} \pm 15 \text{ percent}$$

Another person of the same age, but from an arctic station, eating 300 calories less food and having a skinfold thickness of only 3 cm. would have an expected Recovery Time of:

$$Y=89-10 \text{ seconds}=79 \text{ seconds}\pm 15 \text{ percent}$$

These are the expected values—the norms for these subjects—the *controls* from which to compare and interpret the observed values gotten from actual examination of these individual men.

It will now be clear that had we made further groupings of relevant discontinuous variates and/or selected independent variables which together accounted for a still larger portion of the variability in the dependent variable, we would have reduced the "error of estimate" of our calculated expected value; that is, we should have increased the precision of our control.

How does this work in practice? Let us suppose that in our hypothetical survey, information was recorded which would enable a classification of individuals into two occupational groups such as sedentary and active. The case histories then might yield data as in table 4.

TABLE 4

Typical Survey Data

Occupational group	Age (yr.)	Weight (kg.)	Skinfold (cm.)	Calories	Heart recovery time		
					As measured	As expected	Percent differential
Sedentary	25	68	3. 4	3, 400	105	110	+5
Active	30	65	2. 7	3, 800	55	62	−11
Sedentary	35	70	3. 0	3, 800	75	67	+12
Active	38	75	3. 5	3, 700	40	76	−47
Active	30	70	3. 0	3, 500	50	90	−44
Averages:							
Sedentary $n=25$					93	90	+3
Active $n=25$					75	90	−28

To give precise interpretation to these data we must use the "error of estimate" which was ±15 percent. One way of doing this is to calculate the Least Significant Difference between *expected* and *measured* times. The difference necessary between observed and expected values for any one person may be taken as

$$\text{Error of estimate } x\sqrt{2}\times t$$

and t for a probability of 95 percent will be approximately two. Thus we may calculate that if the observed Recovery Time differs from the measured time by at least ±42 percent (i. e., $\pm 15\times\sqrt{2}\times 2$), the difference is not due to chance variation. For groups of individuals the necessary differences will be calculated as $\left(\frac{\pm 15}{\sqrt{n}} \times \sqrt{2} \times 2\right)$ which for 25 persons per group would be ±7.5 percent.

From table 4, then, we would have evidence that exercise had a significant beneficial effect on physical fitness of most individuals, regardless of age, weight, fatness, or appetite; and, in groups as a whole, exercise is undoubtedly beneficial. Further examination of exceptional cases might reveal reasons for their reactions.

In another instance we might be concerned with interpretation of a resurvey of areas where compulsory fortification of flour had been instituted. We wish to know whether as a consequence there was any change in nutritional status or physical health among individuals of these populations measurable by our criteria. The mean "year" effect was not significant. However, there might be a pronounced effect on individuals who by their diet selection had previously consumed a ration deficient in those nutrients now added to the flour. Our method of analysis would assist in disclosing such changes.

Or we may wish to include among "stations," areas where water fluoridation was involved or where a milk campaign has been carried out. Differences in the group means give us the basis for correction figures to apply to the general average of the dependent criterion used. It is merely an extension or modification of this method, to provide by previous design of trial, groupings to differentiate the effects of *imposed* experimental treatments such as would be represented by a factorial arrangement of the treatments in planned experiments with laboratory animals.

For other dependent variables to be studied we might have such data as: O_2 consumption, N_2 balance, Ca balance, a quantitative record of night blindness, numerous blood analyses or urinary excretion data for vitamins, etc. The dependent variable may be any criterion which is pertinent, provided only that it can be expressed quantitatively, and that biologically it is affected by the independent variables chosen. We have found the method highly flexible and at the same time efficient in the critical analysis and evaluation of nutritional research data.

And now three comments. The first is that the data herein presented are hypothetical, though reasonably plausible. The second is from the experience of investigators generally: statistics is helpless as a tool to improve faulty data. The last and most important is from the wisdom of R. A. Fisher, the father of modern statistical methods as applied to biological data: if your statistics does not agree with biology, suspect the statistics.

II. Evaluation of Protein Adequacy

Biological Value of Proteins and Amino Acid Interrelationships

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There are many advantages that would result from the successful evaluation of food proteins from their content of amino acids, particularly the essential amino acids and those nonessentials whose obligate precursors include an essential amino acid. These advantages would include not only an appraisal of nutritional quality in a single term, but also a clear indication of the essential amino acids limiting the nutritional quality, and therefore of the ways of combining foods into diets to ensure the most effective supplementation. Another advantage of the chemical method of appraisal is the saving of time, since the microbiological analyses of the amino acid contents of proteins can be carried out in a few days, where a biological appraisal would take several weeks.

The discussion in this paper will be concerned only with the relationship between the amino acid content of the proteins of foods and protein utilization in metabolism. Insofar as known, the digestibility of food proteins (nitrogen) is not related to the amino acids they contain, as digestibility is commonly measured. Interest in measuring the digestibility of human diets is so much out of vogue that in the latest edition (1952) of Sherman's classical book, *Chemistry of Food and Nutrition*, the only quantitative information on digestibility of food nutrients is taken from Atwater's compilation published over 50 years ago (*4*). In this compilation the average coefficients of apparent digestibility of protein for different classes of foods are: 97 for animal foods, 85 for cereals and breadstuffs, 78 for dried legumes, 83 for vegetables, 85 for fruits, and 92 for an average mixed diet. From the fact that the metabolic fecal nitrogen, excreted in porportion to the total dry matter consumed, is charged against the dietary nitrogen in the usual methods of computing digestibility, it follows that in general the coefficient of apparent digestibility of protein in different foods will be directly correlated with the protein content of the food on the dry matter basis (*7*).[1] Other relations in human nutrition may be found between the proximate composition of foods and the digestibility of the contained nutrients if, and when, this general problem attracts the interests of investigators as it has in animal nutrition (*42*).

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[1] Italic figures in parentheses refer to Literature Cited, at end of article.

Chemical Evaluation of the Nutritional Quality of Food Proteins

The present status of the nutritional classification of the proteinogenous amino acids for the rat, upon which the chemical evaluation of protein quality in nutrition must largely be based, may be presented as in table 1, representing a modification of one recently published by Rose, Oesterling, and Womack (*41*). An important conclusion to be drawn from this table is that in the nutritional evaluation of proteins by their amino acid composition, it is not sufficient to determine the content only of the essential acids. Cystine and tyrosine should also be determined, since their occurrence in dietary proteins up to certain amounts dependent upon the tissue needs for them, will decrease the proportions in the dietary proteins of their precursors, methionine and phenylalanine, respectively, necessary for adequate nutrition. Many published analyses for the essential amino acids only in proteins and food materials are not adequately suited for nutritional evaluation for this reason. The situation is more serious for cystine than for tyrosine, since methionine deficiencies of food proteins seem to be of frequent occurrence (though perhaps not so frequent in human nutrition), while deficiencies of phenylalanine have not as yet been revealed, to the author's knowledge.

TABLE 1

THE NUTRITIONAL SIGNIFICANCE OF THE PROTEINOGENOUS AMINO ACIDS FOR THE GROWING RAT

Essential amino acids	Semi-dispensable amino acids	Nonessential amino acids
Lysine	Arginine [1] ←	→ Proline [4]
Tryptophane	↑	→ Glutamic acid [4]
Histidine		Aspartic acid
Phenylalanine →	Tyrosine [2]	Hydroxyproline
Leucine		Alanine
Isoleucine		Glycine
Threonine		
Methionine →	Cystine [3] ←	Serine
Valine		

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[1] Arginine can be synthesized by the rat but at a rate not sufficient for maximum growth.

[2] Tyrosine is synthesized in the body only from phenylalanine and may replace roughly one-half of the phenylalanine requirement, but has no growth effect in the *absence* of phenylalanine.

[3] Cystine can be synthesized by the rat (in any appreciable amount at least) only from precursors including methionine. It can apparently replace methionine in the ratio of 1 molecule of cystine to 2 molecules of methionine, to an extent that has not been satisfactorily determined. It has no growth effect in the *absence* of methionine.

[4] Glutamic acid and proline can serve individually as rather ineffective substitutes for arginine in the diet. This function is probably not shared by hydroxyproline. Under conditions otherwise permitting rapid growth, the biosynthesis of glutamic acid may not be sufficiently rapid to cover requirements.

The mouse (*5, 43*) and the pig (*26*) resemble the rat in their qualitative amino acid requirements, as does also the human infant, insofar as its requirements have been studied, except that arginine and histidine are nonessential according to Albanese (*1*).

The author has developed elsewhere (*29*) the concept that the amino acid requirements of the growing animal are determined in the last analysis by the amino acid composition of the tissue proteins formed during growth, superimposed upon the amino acid requirements for the replacement of the endogenous losses of nitrogenous material, i. e., for the maintenance of the *status quo.* The striking similarity in the amino acid contents of homologous tissues of a wide range of animal life is illustrated by muscle tissue of which Beach, Munks, and Robinson (*6*) conclude from their extensive study of the subject:

"The protein mixture which makes up voluntary muscle tissues is similar in Mammalia, Aves, Amphibia, Pisces, and Crustacea, with respect to ten of the amino acids. Since muscle tissues of these various classes of animals do not differ widely in their amino acid patterns, the findings support the belief that the same or closely similar amino acid composition of muscle proteins is repeated throughout the animal kingdom."

This concept suggests that the amino acid requirements of the various species of animals should be similar, except for differences in the relative growth of the integumental tissues, hair, wool, horn, etc. Furthermore, if the physiological efficiency in the conversion of dietary protein to tissue protein (and other nitrogenous tissue constituents) does not vary greatly from one species of animal to another, then the nutritional values of the proteins in different food materials should be similar and should possess the same limiting essential amino acids. The plausibility of this assumption rests on the apparent similarity in the biosynthetic reactions of animal tissues.

The classification of the amino acids occurring in proteins on the basis of their biological significance was a most important advance in the nutritive evaluation of proteins by chemical analysis, since attention could be centered, perhaps exclusively, on those amino acids ". . . which cannot be synthesized by the animal organism, out of the materials *ordinarily available*, at a speed commensurate with the demands for *normal* growth," Rose's definition of an indispensable dietary component (*40*).

But even so, the precise evaluation of proteins by such means was not at hand. Proteins could be compared by graphing their amino acid "contours" or "spectra," but 1 percent of one amino acid in a protein obviously possesses a different nutritive significance than 1 percent of another. What was missing was a yardstick of comparison, represented by the amino acid content of a protein or protein mixture with perfect availability in digestion and in metabolism.

Kühnau in 1949 (*21*) took human milk as the standard or yardstick for the evaluation of proteins in human nutrition. Summation of the percentages of the essential amino acids in the mixed proteins of human milk, and also those of arginine, cystine, and tyrosine, results in a value of 67.5 (see table 2). For egg proteins, the summation is 66.7. Taking human milk protein as 100, a value of 99 is obtained for egg; this is called the "total value" (gesamte Wertigkeit). But this credits the latter proteins with concentrations of amino acids in excess of those occurring in the standard, excesses that presumably contribute nothing to the inherent nutritive value of egg proteins. When the summation is repeated, disregarding these excesses, and the sum divided by 67.5, a value of 90 is obtained. This is called the "pure value" (reine Wertigkeit). The difference between the two values, 9, is the "supplementary value" (Ergänzungswertigkeit), and is presumably available for supplementary relations with other proteins that may be deficient in the amino acids occurring in excess in egg proteins.

TABLE 2

CALCULATION OF NUTRITIVE VALUE OF PROTEINS BY THE METHOD OF KÜHNAU

Amino acids	Content per 16 grams of nitrogen		Excess of test over standard protein
	Human milk	Egg	
	Gram	*Gram*	*Gram*
Valine	9. 9	7. 3	------------
Leucine	10. 2	9. 2	------------
Isoleucine	7. 6	8. 0	0. 4
Threonine	5. 0	5. 9	. 9
Methionine	2. 3	4. 1	1. 8
Cystine	3. 4	2. 4	------------
Phenylalanine	5. 9	7. 3	1. 4
Tyrosine	5. 1	4. 5	------------
Tryptophane	1. 9	1. 5	------------
Arginine	5. 0	6. 5	1. 5
Histidine	2. 7	2. 1	------------
Lysine	8. 5	7. 9	------------
Sum	67. 5	66. 7	6. 0
Gesamte wertigkeit	100	99	------------
Reine wertigkeit	------------	$\frac{66.7-6.0}{67.5}=90$	------------
Ergänzungswertigkeit	------------	99—90=90	------------

It is to be noted that Kühnau's method does not give equal weight to the amino acids considered in deriving his final values. Rather, each amino acid is weighted in accordance with its quantitative occurrence in the standard proteins.

Using a very similar method to that of Kühnau, Oser (*38*) recommends the computation of an "essential amino acid index," in which, following the recommendation of Mitchell and Block (*31*), he selects whole egg protein as a standard. The test-protein : egg-protein ratios are computed and expressed as percentages; all percentages over 100 are then reduced to 100, thus disregarding proportions of all amino acids in excess of those occurring respectively in the standard protein, following the plan of Kühnau. A geometric mean is then taken of these ratios, equal to the anti-logarithm of the mean logarithm of the ratios. To avoid the use of negative logarithms, all egg-protein ratios less than 1 are considered equal to 1. Oser does not consider tyrosine in his calculations, but he does include cystine and arginine. The geometric mean is preferred to the arithmetic mean, since it possesses certain advantages in the averaging of ratios.

Mitchell and Block (*31*) approached the subject of the chemical evaluation of proteins in a different way. Kühnau and Oser computed values that integrated the essential amino acid contents of proteins (together with cystine, or cystine and tyrosine) in terms of the corresponding values of a standard protein chosen for its excellence in nutrition. By this method of computation the deficiencies that limit the nutritive values of proteins tend to be obscured if in other respects the amino acid composition compares well with that of the chosen standard. The idea of Mitchell and Block was to judge the nutritive adequacy of a protein by the severity of its limiting essential amino acid deficiency. The standard protein source was whole egg which had been shown to be nearly perfectly utilized in digestion and in metabolism for the growing rat (*33*), for the mature rat (*11*), for the dog (*2*), and for adult man (*17*). Furthermore, Mitchell (*29*) has shown that the growth-promoting value of whole egg protein, when carefully prepared to avert heat and other damage, is not improved for the growing rat by supplementation with any of the essential amino acids except lysine, which induced a 3 percent increase (statistically significant) in body weight gain in a 28-day feeding period. The absence of any nutritionally unavailable essential amino acids in whole egg proteins is demonstrated by the fact that substitution of whole egg for starch (at low levels) in a nitrogen-free diet fed to rats does not appreciably increase the output of urinary nitrogen (*11*).

Proceeding on this basis, Mitchell and Block compared the contents of the essential amino acids in single proteins and in the mixed protein of foods with those of egg, by computing the percentage deviation for each amino acid. The nutritive value of the protein was expressed as a "chemical score," equal to the greatest percentage deficit in an essential amino acid in the protein or protein mixture under scrutiny. This score, originally, was set as 100 for any protein or protein mixture completely lacking in any one essential amino acid, but later and better (*31*), the chemical score was taken as 100 minus the greatest percentage deficit, in other words, the smallest percentage of an essential amino acid based on its content in egg protein.

It should be pointed out that the chemical score as defined by Mitchell and Block measures the value of proteins for growth only, since it is based upon the concept that the complete lack of an essential amino acid renders a protein completely unavailable for the synthesis of body proteins. For maintenance of the *status quo* of the tissues it is known that proteins incomplete in one or more of the essential amino acids may nevertheless be used for the replacement of endogenous losses of nitrogen (*24*, *25*). For this reason, the integration of egg-protein ratios of essential amino acid (plus tyrosine and cystine) contents gives a better measure of the nutritive value of proteins for maintenance and growth, since growth cannot be studied alone.

On the other hand, the amino acid integration methods of Kühnau and of Oser imply that the only amounts of the essential amino acids in a given protein that are unavailable in anabolism are the amounts present in excess of those in the standard protein. However, to some extent the deficit in that amino acid limiting the value of the protein for growth will limit the utilization of all other essential amino acids. For example, if lysine is the limiting amino acid of a food protein that contains only one-half of the amount of lysine in the standard, then for purposes of growth only, the utilization of all other essential amino acids would be limited to 50 percent of the amount present in the standard. Due to the utilization of essential amino acids for other purposes than protein synthesis, this general limitation of amino acid utilization would not be as severe as this. The exact extent of general limitation is not known; presumably it would be a function of the ratio of nitrogen used for growth to that used for maintenance, which in turn depend upon the level of protein in the diet.

In testing the biological validity of chemical methods of evaluating the nutritive quality of proteins, a modified essential amino acid index will be used, keeping in mind the shortcomings of the Oser index. In computing the revised index, arginine is omitted from consideration, because it is neither essential in the strict sense of the term, nor does it have an obligate precursor among the essential amino acids, as tyrosine and cystine do. Furthermore, the rate of its synthesis in the body, from proline, glutamic acid and possibly other precursors, with reference to the rate of growth of the animal may be adequate or inadequate for normal growth, depending upon the species and stage of growth. On the other hand, tyrosine and cystine are included because their presence in the protein, but only up to the amount present in the standard proteins (of whole egg), will diminish the requirements for phenylalanine and methionine, respectively. Following the practice of Kühnau and of Oser, contents of amino acids in excess of those present in the standard protein are disregarded. The geometric mean of the revised egg ratios (expressed as percentages) is used in accordance with the practice introduced by Oser, and percentages less than 1 are considered to be 1 in order to avoid negative logarithms and zero indices.

As an example of the calculation, consider lactalbumin, and table 3.

TABLE 3

Computation of Modified Essential Amino Acid Index

Amino acids	Amino acid content per 16 grams nitrogen		Egg ratio	Corrected ratios	Logarithms of corrected ratios
	Whole egg protein	Lactalbumin			
	Gram	*Gram*	*Percent*	*Percent*	
Histidine	2. 4	2. 3	96	96	1. 98227
Lysine	7. 0	10. 5	150	100	2. 00000
Phenylalanine	6. 3	5. 0	79	--------	----------
Tyrosine	4. 5	5. 3	118	--------	----------
Phenylalanine plus tyrosine	10. 8	10. 3	95	88	1. 94448
Tryptophane	1. 5	2. 5	167	100	2. 00000
Methionine	4. 0	2. 6	65	--------	----------
Cystine	2. 4	4. 0	167	--------	----------
Methionine plus cystine	6. 4	6. 6	103	78	1. 89209
Threonine	4. 3	6. 0	140	100	2. 00000
Leucine	9. 2	12. 1	132	100	2. 00000
Isoleucine	7. 7	7. 5	97	97	1. 98677
Valine	7. 2	6. 6	92	92	1. 96379

Average logarithm	1. 97438
Modified essential amino acid index	94

This protein contains an excess of tyrosine (18 percent) and of cystine (67 percent) as compared with egg proteins. It is, therefore, credited only with 4.5 percent of tyrosine and 2.4 percent of cystine in computing the percent of phenylalanine+tyrosine (88 percent) and of methionine+cystine (78 percent). The essential amino acid index of this protein will therefore be the geometric mean of the following numbers: 96, 100, 88, 100, 78, 100, 100, 97, and 92, which is 94. The limiting amino acid of lactalbumin, according to these analyses, is methionine and the chemical score is 78.

Correlation of Chemical Evaluation of Proteins With Their Biological Value

The validity of the modified essential amino acid indices and the chemical scores of the protein mixtures of foods will be assessed by correlating them with the corresponding biological values determined

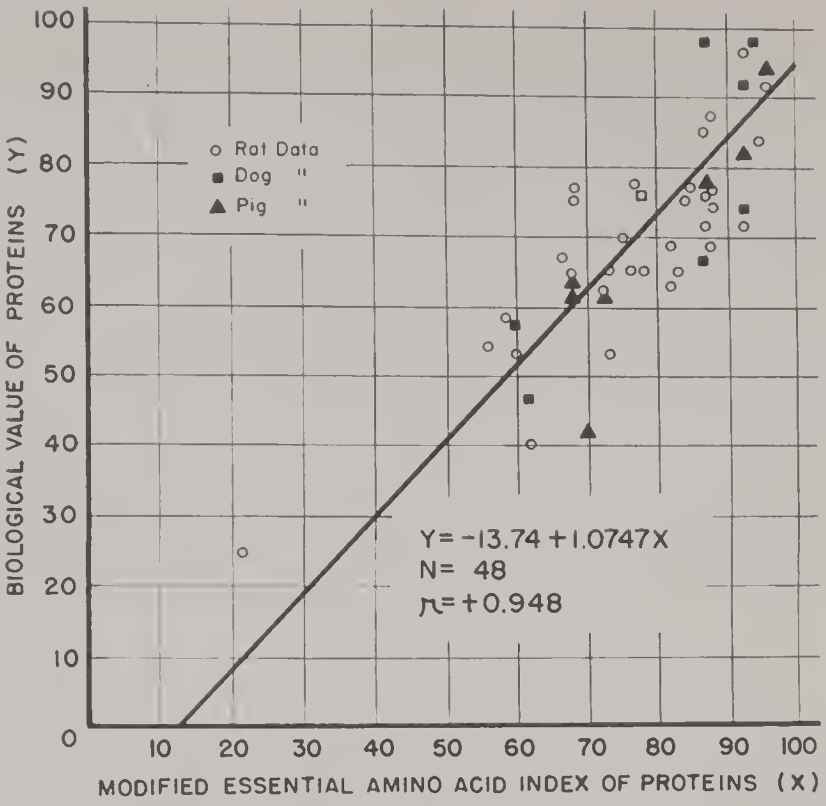

Figure 1.—Relationship between modified essential amino acid indices and the biological values.

by the nitrogen balance method. The biological value of a protein in its ultimate terms may be defined as follows:

$$\text{B.V.}=100\times\frac{NB+MFN+EUN}{NI-(FN-MFN)}$$

in which NI is the nitrogen intake, FN the fecal nitrogen, NB the nitrogen balance, MFN the metabolic fecal nitrogen, and EUN the endogenous urinary nitrogen, all for the same period of time.

A total of 48 food proteins have been selected for which apparently satisfactory analyses of the essential amino acids (plus cystine and tyrosine) and apparently satisfactory biological values are available. Most of the biological values were obtained with growing rats, but the selection also includes 7 values secured with growing pigs and 8 values for (presumably) growing dogs. The references yielding the chemical data and most of the biological data used in this correlation have been cited elsewhere (*28*). The recently published biological values for dogs of Murlin *et al.* (*35*) have been added.

Figures 1 and 2 present graphically the relationship between the modified essential amino acid indices explained above, in the first case, and the chemical scores of Block and Mitchell (*9*) in the second, and the biological values. The correlations in both cases are high, 0.948 and 0.833, respectively. The difference between these coefficients is highly significant statistically ($P=ca.\ 0.003$).

In examining these figures it will be noted that the rat, pig, and dog data are apparently homogeneous in conformance with expectations.

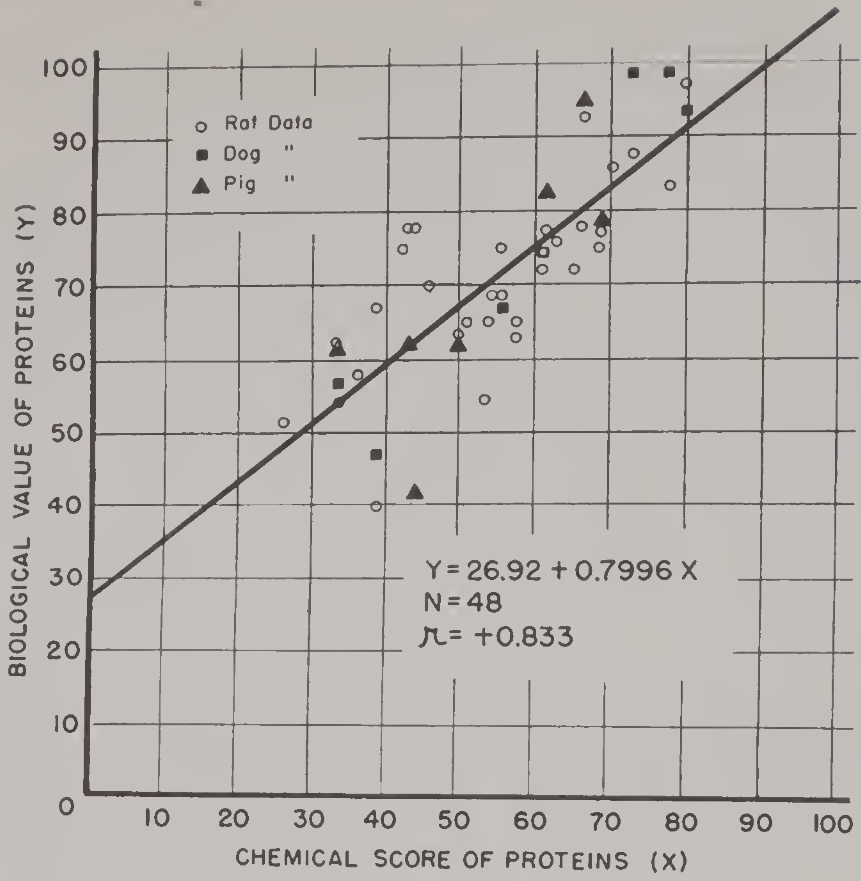

FIGURE 2.—Relationship between chemical scores and the biological values.

Information concerning the utilization of proteins by growing children is sparse and discordant. What little that has been found in the literature has been reviewed by the author elsewhere (*27*). The following biological values for food proteins reported by Edelstein and Langstein (*15*) are worthy of note: cow's milk 73, woman's milk 88, lactalbumin 87, and casein 73.

Two possible explanations for the difference in the sensitivity of the two methods for the chemical evaluation of proteins occur to the author. On the one hand, it may be partially an expression of the fact that in the integration of nine amino acid ratios between test proteins and standard protein in the computation of essential amino acid indices, a compensation of errors in amino acid analysis will result in a more accurate criterion of the desired significance than a chemical score based upon only one ratio. On the other hand, the essential amino acid index may take proper cognizance of differences in the amino acid requirements of maintenance and of growth, while the chemical score is based upon prevailing conceptions of the amino acid requirements for growth only.

Evidently the error of prediction of the biological value of a food protein (as this technique is commonly employed by the procedure developed in this laboratory) is considerably less when the prediction is made on the basis of the essential amino acid index (modified) than when made on the basis of the chemical score. It may be shown by statistical analysis that for a protein with a biological value of 60, for example, the standard error of estimate would be about 0.85 based upon the essential amino acid index, and about 1.77 based upon the chemical score.

It is really a matter of surprise that the chemical assessment of the nutritional value of a protein is so highly correlated with the biological assessment in view of the known errors in the chemical analysis of proteins for amino acids so well illustrated by the discrepancies in the analysis of identical proteins among cooperating laboratories in the Rutgers investigation (*3*). Biological methods of assessment are also subject to errors of appreciable magnitude (*32*). Coupled with these technical errors is the fact that essential amino acids serve so many purposes in the body other than protein synthesis and endogenous replacements, such as transmethylation and detoxication reactions, methyl group synthesis, bile acid synthesis, nicotinic acid synthesis, hormone production, and hepatic lipotropic reactions.

A peculiar advantage of methods of appraising the nutritive value of proteins by their contents of the essential amino acids and of those semidispensables whose obligate precursors are essential amino acids, is the fact that they can so accurately identify the essential amino acids that limit biological utilization. Of 15 proteins and protein sources for which the primary amino acid limitation has been determined by feeding experiments, mostly with rats, as well as by computation of the chemical score, the verdicts of both methods are in agreement (*28*). The second limiting amino acid of corn proteins has been definitely identified by both methods as tryptophane, while that of zein is clearly lysine.

Calculations of the egg ratios of proteins by the method illustrated in table 3 permit predictions of supplementary relations among proteins. Obviously proteins with the same limiting amino acid cannot supplement each other in nutrition. Animal muscle proteins are deficient in methionine-cystine according to such computations as are also pea proteins; when combined, the growth-promoting value of the combination is intermediate between those of the two individual foods; but that true supplementation had not occurred is revealed by the failure of the meat supplement to cure the hepatitis induced by the methionine deficiency of the pea ration; methionine supplementation effected a cure (*22*). On the other hand, the combination of two food protein mixtures deficient in different amino acids will always result in mutual correction of the respective deficiencies. The extent of supplementation may be slight, as in a meat-egg combination (*19*), with moderate methionine and lysine limitations, or it may be marked as in a meat-white flour combination (*20, 33*), the latter protein food being markedly deficient in lysine, while the former is abundantly supplied with it.

Application to Adult Human Nutrition

This symposium is mainly concerned with the evaluation of the nutritional adequacy of a normal adult human population. The material that has been presented thus far, however, was secured with the lower animals, mainly the laboratory rat, and during the period of growth, not maturity. This has been necessary in the establishment of the principles of protein evaluation in nutrition, because the large mass of available information relates to the lower animals. What data on adult humans are available are so discordant as to belie their validity. When the same laboratory over the years has reported biological values for adult humans for whole-egg proteins ranging from 65 to 94, for peanut flour protein values ranging from 42 to 83, and for white flour protein values ranging from 42 to 75, it is apparent that human variability in the utilization of dietary protein can hardly be the main cause of discrepancy. The biological values for adult man of plant and animal proteins reported by Lintzel (*23*), indicating a marked superiority of the proteins of rye and wheat over those of milk, meat, and eggs is incomprehensible on the basis of the amino acid contents of these protein mixtures and of results secured with the lower animals. The conclusion seems inevitable that the technique of determining the biological value of food proteins in the human has not been sufficiently standardized, until recently at least. The difficulty in inducing human subjects to consume the low-protein diets required to measure the body's contribution to the urinary nitrogen has been a serious deterrent to this field of research. It is to be hoped that this difficulty can be largely obviated by the use of the creatinine excretion in the urine in assessing the endogenous urinary nitrogen as suggested by Murlin and others (*36*) and confirmed by Bricker and Smith (*13*), and later for dogs by Murlin, Hayes, and Johnson (*35*). In continuing this discussion it must be assumed that the latest results from the Universities of Rochester and of Illinois are comparable and satisfactory. The biological values for adult humans reported by the Harvard group (*18*) cannot be used in this connection because the requirements relate to mixed diets, the amino acid contents of which are not given and cannot be satisfactorily calculated from available information.

Table 4 summarizes the biological values of a series of proteins for the adult and the growing rat, for the adult dog, and for adult man, taken mainly from the Rutgers cooperative experiment (*3*). The points of agreement and disagreement betwen the adult rat and adult man are mainly traceable to the greater dominance of keratin synthesis in a species covered with hair undergoing continuous molt (*16*). The results for the dog, like those for the adult rat, reveal a relatively low value for proteins deficient in methionine-cystine (beef muscle, casein, peanut flour, and soy flour) and, for the adult rat, a high value, relative to adult man, of the proteins deficient in lysine (wheat gluten and white flour).

TABLE 4

THE BIOLOGICAL VALUES OF PROTEINS FOR ADULT RATS, DOGS, AND MEN AS COMPARED WITH THOSE FOR GROWING RATS

Protein source	Growing rat (*30*)	Adult rat (*11, 30*)	Adult dog (*34*)	Adult men (*12, 17*)
Egg albumin	97	94	89	91
Whole egg (commercial)	87	82	86	94
Beef muscle	76	69	57	67
Wheat gluten	40	65	--------	42
Casein	69	51	68	68
Peanut flour	54	46	61	56
Cow's milk	[1] 90	[2] 86	--------	[1] 74
White flour	52	[3] 65	--------	41
Soy flour	75	49	--------	65
Whole egg, laboratory prepared	96	99	--------	--------

[1] Fresh milk.

[2] Dried defatted milk.

[3] Unpublished.

The main purpose of the table, however, is to reveal the correlations between the biological values for the growing rat and the adult rat ($r=0.658$), between the growing rat and adult man ($r=0.921$), and between the adult rat and adult man ($r=0.604$). Among this series of nine proteins, man resembles the growing rat more closely than he does the adult rat in his metabolic utilization of food proteins. With this small series of paired variates, significant differences between correlations cannot be established. A corollary from this situation, if it may be assumed to have a general significance, is that the biological value of proteins in adult human nutrition may be satisfactorily predicted, as it is with the growing rat, from the essential amino acid indices of proteins. Of the same significance is the fact reported by Bocobo *et al.* (*10*) that a high correlation exists ($r=0.811$) between the amino acid composition of whole egg proteins and the average amino acid composition of human muscle, heart, and liver, and that the correlation with human tissues diminishes as food proteins of lower biological value are considered, the coefficient being 0.791 for casein, 0.516 for wheat gluten, and 0.455 for the proteins of whole corn. The essential amino acid index of the proteins of human muscle is 82, as compared with 87 for animal muscle; the chemical score is 67 compared with 69, and is determined by the combined content of methionine and cystine.

The direct correlation of biological values of food proteins for adult humans and the corresponding modified essential amino acid indices is shown in table 5. The foods are arranged in the order of decreasing amino acid indices. The product moment correlation coefficient is +0.815 and the regression equation of biological value (y) on modified essential amino acid index is:

$$y = -15.55 + 1.0363x.$$

On referring to figure 1 it will be seen that this regression equation is nearly the same as that for the similar correlation for growing rats, dogs, and pigs.

TABLE 5

CORRELATION BETWEEN THE BIOLOGICAL VALUES OF FOOD PROTEINS FOR ADULT MAN AND THEIR MODIFIED ESSENTIAL AMINO ACID INDICES (EAAI)

Protein source	Modified EAAI	Biological value	Food source	Modified EAAI	Biological value
Whole egg	100	91	Yeast	81	52
Milk	94	74	Beef muscle R	78	67
Egg albumin R	93	91	Sunflower seed	73	62
Whole egg R [1]	87	94	Wheat gluten R	62	42
Casein R	87	68	White flour	60	41
Soy flour	84	65	Peanut flour R	56	56

[1] The whole egg preparation in the Rutgers study was definitely impaired in biological value for the growing, as well as the mature, rat (*30*).

Note. Those foods designated R were those tested in the Rutgers cooperative study (*3*). The EAAI were computed from the analyses given in that report and the corresponding biological values are those reported by Hawley *et al.* (*17*). All other indices were computed from the analyses given by Block and Bolling (*8*). Other biological values than those of Hawley *et al.* were taken from Bricker, Mitchell, and Kinsman (*12*): milk, soy flour, and white flour; from Bricker and Smith (*13*); milk- and sunflower seed flour; and Cremer and Lang (*14*): yeast.

It may be concluded tentatively that the modified essential amino acid index applies in a fairly satisfactory manner in assessing the nutritive evaluation of food proteins, to the adult human. The fact that differences in the apparent digestibility of food proteins do not seem to interfere seriously with correlations such as these must indicate that the proportions existing among the absorbed essential amino acids (plus cystine and tyrosine) are not greatly different from those existing in the total proteins. When measurements of the mean requirements of the adult human for the essential amino acids plus cystine and tyrosine are determined and published for critical scrutiny, it will be possible to base essential amino acid indices on a firmer basis than can now be done.

In human nutrition the damaging effect of heating and processing foods for consumption may be a disturbing factor in any chemical method of assessing protein utilization in metabolism (*37*). Prolonged storage of food may also adversely affect protein values.

Conclusions

The dependence of the biological value of proteins, determined under standardized conditions, on the content of the proteins in the essential amino acids plus those semi-dispensable amino acids, cystine and tyrosine, with obligate precursors among the essential amino acids, is a close one for growing monogastric animals. The correlation between the biological value of proteins and the chemical appraisal of their nutritive value by an essential amino acid index modified from that of Oser—and using whole egg proteins as a standard—is so high ($r=0.948$) as to permit an accurate estimate of the former from the latter. For the human child, the situation may be different, in the sense that for a given food protein, biological utilization may be less complete than for the growing rat, dog, or pig.

Maintenance nutrition in all animals involves different amino acid demands than does maintenance and growth, particularly for those animals maintaining a heavy coat of hair, fur, or feathers. In such cases, the requirements for keratin synthesis dominate the demands for amino acids. Since adult man is not in this class of animal life, keratin synthesis is not an important item in his protein nutrition. In fact, there is good evidence that the proportionate requirements of essential amino acids for the maintenance of nitrogen equilibrium in man simulate those required by growing animals for maximum nitrogen retention. To the extent that this is true, the biological value of food proteins for adult man may be predictable from the essential amino acid indices (observed correlation in 12 cases cited=0.815) by the same equation, or a slightly modified one, that applies so well to the protein nutrition of the growing rat.

When the essential (and semi-dispensable) amino acid requirements for maintenance, and for maintenance and growth have been determined with satisfactory accuracy, they can be used, in place of some reference protein of superior nutritive value, in computing essential amino acid indices. Such an ideal situation has not as yet been realized for any animal under any conditions in the author's opinion. In the case of man (*39*), published requirements admittedly are not average results at all, and hence not the most probable estimate of requirements as the term is ordinarily used.

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The Rat Repletion Method

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In discussing the Rat Repletion Method as a means of evaluating protein adequacy, the first point to emphasize is that we regard this method more as a nutritional principle than as a formal method. In short, it is essentially a procedure which enables a protein-depleted animal quickly to reconstruct tissue proteins that have undergone diminution during a preliminary period of general protein depletion. As a method, however, we have found it helpful for the study of various aspects of protein metabolism, including the evaluation of the protein quality of foodstuffs. Most of this discussion will deal with the latter question, with the reservation that the procedure is still to be looked upon as flexible and subject to further modifications. We shall continue to emphasize its use in terms of the broad principle of nutritional repletion even while alluding to it as a method. Some 10 years of experience with it have convinced us of its value in a number of ways, but claims for its value must obviously be supported by evidence that it possesses qualities of reliability, ease of performance, and a reasonable quantitativeness. This report describes a few of the ways in which we have used it and a few of the results we have obtained, both as a method and as a means of studying broader nutritional problems of amino acid utilization. The blibligrophy (p. 36) lists our own and other studies pertinent to this brief exposition of the method and its uses.

There are a number of reasons why we believe that the principle of repletion is sound, nutritionally, as it is applied in the Rat Repletion Method. Primarily, the method represents a type of bioassay employing an animal which is acutely responsive to inherently urgent needs for nutritional rehabilitation. It also has the advantage of speed as compared, for example, with the rat growth method in that an assay can often be accomplished in 7 days, thus avoiding the use of cages over a long period of time. It uses mature animals with established food habits and with relatively mature enzyme and endocrine systems. It is readily adaptable to other procedures, such as paired-feeding techniques, nitrogen balance tests, carcass analyses and the like. Because the rats are mature they can be conditioned to drink solutions, such as amino acid solutions or protein hydrolysates, while their other nutritional needs are being satisfied by the ingestion of a nonprotein ration supplying roughage, calories, salts, and vitamins. They can also be used for the study of problems of parenteral nutrition, including the evaluation of protein hydrolysates, fat emulsions, and therapeutic procedures as, for example, the actions of hormones and electrolytes administered by intravenous, intraperitoneal or subcutaneous routes.

Some of the disadvantages of the method are obvious. Of these probably the greatest is that the test subject is a rat rather than a human subject. The time required for protein depletion is also a disadvantage, particularly when the method is used only occasionally, but this disadvantage can be easily overcome by a rapid depletion of 12 days, as Frost and Sandy have shown. Thus it is a simple procedure to induce a loss of weight of 35 percent in adult rats; these rats can then be used satisfactorily for purposes of protein repletion. Older rats are also somewhat more expensive than young ones, but it might be pointed out that young rats can be used for growth tests and then used later for depletion-repletion purposes. Furthermore, some workers have used the animals for a second depletion and repletion with reportedly satisfactory results.

Probably the greatest value of the method stems from the extreme sensitivity of the protein-depleted rat to essential amino acid deficiency while being subjected to the stress of rapid protein repletion. For example, when such an animal is fed a ration adequate in all dietary essentials except a single indispensable amino acid, the characteristic response is a depression of appetite and a failure to gain weight. This response is quickly altered, however, when the missing essential amino acid is added to the food or drink. Similarly, if the animal is offered a ration with one or more essential amino acids either present in suboptimal amounts, or in a form unavailable for enzymic action in the digestive tract, food acceptance is either impaired or the simultaneous absorption and utilization of the essential amino acids is hampered. This is probably true for each of the nine amino acids considered as essential for the growth of rats. The response to variations in intake of any one of the indispensable amino acids is remarkably quantitative so that it has been possible to establish minimal daily utilization rates for each of the nine in relation to the others. Because of this sensitiveness with respect to essential amino acid requirements in protein repletion, we were able to demonstrate the importance of the time factor in relation to the need for the simultaneous availability of essential amino acids at the anabolic site in tissue protein synthesis.

Early in World War II we were asked by the Quartermaster Food and Container Institute for the Armed Forces to investigate a variety of problems relating to the protein adequacy of foods and rations as these might be affected by conditions of processing and storage. We undertook the work after having become convinced of the potential usefulness of the principle of protein depletion for the detection of essential amino acid weaknesses of a ration. Obviously the problems to be investigated required a procedure which could operate with a rapid turnover of cage space and could function as a screening method in helping to solve some of the problems of military concern.

Our first task was to ascertain the response of protein-depleted rats to protein repletion when fed a daily ration supplying, in 15-gm. portions, an intake of 216 mg. of nitrogen, 48 calories, and amounts of roughage, vitamins, and salts adequate for effective tissue protein synthesis. As a "standard of reference" protein we chose a mixture of equal parts of lactalbumin and casein and stored a large amount of this for later use. With such a ration, groups of from 6 to 10 rats, in a repletion period of from 7 to 10 days, made average weight gains, regularly, of from 35 to 45 gm. per rat. This performance could therefore be compared with that of similar groups of animals fed rations containing test proteins supplying similar amounts of nitrogen. All animals were kept in individual cages and food wastage was carefully determined so that at the end of the repletion period it was possible to determine protein efficiency in terms of grams gained per gram of protein consumed. Foodstuffs to be assayed were subjected to proximate analysis and were mixed into the "nonprotein" basal ration so as to make all rations equicaloric and containing equal amounts of nitrogen. We could thus compare protein intakes and responses in terms of variations in protein quality. For ordinary purposes of evaluation we found that weight gains served satisfactorily as an index of protein potentialities for tissue synthesis. Later we found that under our experimental conditions optimal protein utilization occurred at a caloric intake of approximately 35 calories per rat per day, using animals with an average size at the beginning of the repletion period of around 150 gm. On a surface area basis this averaged about 1,240 calories per square meter per day.

More recently, therefore, we have used the method under the following conditions:

Adult, male, albino rats (Sprague-Dawley) weighing approximately 200 gm. are subjected to rapid protein depletion by being kept in individual cages and fed on alternate days a "nonprotein" ration supplying 32 calories in each 10 gm. until they have lost 25 percent of their initial weights. They are then ready for the repletion test.

Nonprotein 32-caloric ration (in 10 gm.)

	Percent
Dextrin[1]	69.5
Corn oil (Mazola)	4.0
Alphacel[2]	7.5
Salt mixture	6.0
Vitamin mixture	1.5
Choline (50 percent)	0.9 ml.
Ol. percomorphum	0.45 drop
Water	10.6 ml.

[1] Steinhall No. 4.

Salt mixture[3]	Percent
Calcium carbonate	6. 89
Calcium citrate	30. 97
Calcium phosphate (monobasic)	11. 33
Magnesium carbonate	3. 34
Magnesium sulfate	3. 85
Potassium chloride	22. 40
Potassium phosphate (dibasic)	10. 44
Sodium phosphate (dibasic)	9. 40
Trace salts	1. 38

Trace salts	
Copper sulfate	0. 99
Ferric citrate	92. 31
Manganese sulfate	1. 15
Potassium iodide	0. 29
Potassium aluminum sulfate	0. 66
Sodium fluoride	3. 61
Zinc chloride	0. 99

Vitamin mixture	
Nicotinamide	0. 4
Calcium pantothenate	0. 2
Pyridoxine hydrochloride	0. 1
Riboflavin	0. 2
Thiamine hydrochloride	0. 1
Sucrose	99. 0

Material to be tested.—Proximate analysis is done in order to determine the amount of the test substance needed to supply 216 mg. of nitrogen. Correction for calories in the carbohydrate and fat is made by removal of dextrin and corn oil in equivalent amounts and the ration fed in the amounts necessary to supply 216 mg. of nitrogen daily.

A few examples will be given to illustrate the types of problems with which we dealt during the war years. In addition, references to the papers published by us in which the Repletion Method was used and the names of the collaborators are appended. In these papers are described in more detail many of the problems with which we have been concerned.

One of our early undertakings was the determination of the possibly adverse effects upon protein quality of prolonged storage at high temperatures of food materials and components of rations, particularly dehydrated meats, dried milks, dried eggs, and C Rations. For example, we assayed samples of dehydrated beef and pork which had been stored for approximately 22 months at temperatures of 135° F. and 0° F., using samples supplied by Dr. H. R. Kraybill of the American Meat Institute. Although the Repletion Method showed a slight superiority of the samples kept at the low temperatures, nevertheless the meats stored at the higher temperature reacted as high-quality protein, indicating that, if deterioration had occurred, it was at least of a relatively slight degree. Similarly, samples of dried milks also evidenced excellent protein values, even after storage at 100° F. for

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[2] Nutritional Biochemicals Corporation.

[3] Hawk and Oser modification of Osborne and Mendel mixture.

3 months; and specimens of dried eggs which had been stored at 50° to 70° F. for from 6 to 8 months yielded results indicative of a high-protein value.

We also assayed two menus of the C Ration at intervals after storage at 100° F. The ingredients were ground and put into the basal ration at protein levels of approximately 9 percent and the rations were compared with others containing lactalbumin-casein fed at similar protein levels. With both menus the results indicated protein values almost equalling those of the reference protein (from 85 to 90 percent of the performance of the latter). Later assays were performed with C Rations which had been stored at 100° F. for periods of 3 months and 6 months with no evidences of deterioration of their protein quality.

Soon we became interested in the consequences of heat injury to proteins as exemplified by the so-called "browning reaction." In a paper published with Dr. Richard Block we demonstrated the adverse effects of toasting on flour protein and the beneficial action of lysine in restoring the protein value of the heat-injured flour.

In 1947 we were asked to determine the protein adequacy of some samples of cottonseed meal which were submitted to us by the Southern Regional Research Laboratory of the Department of Agriculture. Some of these meals had been prepared according to common commercial methods; others by the "gland-flotation process" at it was being developed by Dr. Charlotte Boatner and her associates. This method employed mechanical fractionation in organic solvents, particularly diethyl ether, for the separation of the pigment glands from the remainder of the cottonseed meal. We mixed each sample into our basal ration at a protein level of approximately 10 percent, and fed the mixtures to protein-depleted rats over a period of 14 days. Our results indicated a definite correlation between repletion performance and the varied conditions of processing and cooking of the meals in the extraction process. Since then these results have been confirmed and extended by others and it is now evident that extensive cooking of these meals, particularly by steam under pressure, causes deterioration in their protein values, especially at temperatures above 200° F.

When the Quartermaster Food and Container Institute became interested in the problem of canned breads we were asked to ascertain the effects of yeast supplementation in relation to protein quality. The breads were: (*a*) one made from white flour containing 2 percent milk solids and the usual enrichment synthetics; (*b*) one similarly mixed and also containing 3 percent of dried brewers yeast; and (*c*) one made from whole wheat flour and containing 2 percent milk solids. After drying and grinding, each bread was mixed into our basal ration to give a protein concentration of approximately 9 percent. The rations were fed to groups of protein-depleted rats over a period of 14 days. As controls, 2 groups of animals were fed the lactalbumin-casein ration, 1 group receiving the latter ration in daily amounts limited to the average consumption of that of the animals eating the enriched white bread ration. Using the results of the enriched white bread as a base line, the repletion method showed that the animals eating the yeast-supplemented bread equalled the performance of those eating the whole wheat bread. Both groups showed an average increase in weight recovery of approximately 33 percent and at least a 20 percent improvement on the basis of protein efficiency. We concluded that yeast supplementation not only added vitamins to the white bread but improved its protein quality as well.

One experiment illustrating the value of essential amino acid supplementation was an assay of corn meal. This specimen had such a low-nutritive value that protein-depleted rats accepted the corn meal ration poorly and lost weight during a 7-day period. Assuming that this was due to several essential amino acid inadequacies, the ration was enriched by the addition of a mixture of all the essential amino acids except leucine. When this ration was fed to the rats which had previously failed to gain weight, the animals accepted the ration completely and made weight gains in the second week comparable to those of the animals eating the control ration. In other words, appropriate supplementation of the corn meal with essential amino acids had converted it into a material of high biological value. Similar results were obtained when a specimen of human globin was supplemented with four essential amino acids in which globin is deficient.

In the cooperative study with the Bureau of Biological Research of Rutgers University pertaining to the nutritive value of six selected protein food sources we applied the method, both in relation to weight gains and to protein repletion with respect to gain in carcass protein, total plasma protein, total hemoglobin, liver protein, and protein efficiency. A summation of ranking orders of performance for all these categories showed the following ratings: lactalbumin-casein, egg albumin, whole egg, beef, casein, peanut, and gluten. It is of especial interest that this ranking order was essentially identical with that established by weight gain figures alone.

Shortly after the end of the war we were able to apply the repletion method to a study of nutritional problems of more basic interest. Among these was a study of the influence of variations in intakes of protein and calories upon the processes of protein repletion. These studies—published under the title "The Dynamics of Protein Metabolism"—have helped, we believe, to clarify some of the questions relating to the metabolic interaction of calories and amino acids. For example, the problem of caloric needs in relation to nitrogen retention in tissue synthesis is confused and controversial. Because some workers have implied that caloric needs take precedence over protein needs in metabolism, the assumption is sometimes made that amino acid utilization in tissue synthesis is almost negligible in the absence of an optimal caloric intake. More recent evidence suggests, however, that in the presence of protein deficiency the tissue avidity for amino acids is so intense that considerable utilization occurs even under conditions of caloric inadequacy. Furthermore, the dynamic state of the labile fat reserves indicates that at least for short periods of time there may be a significant contribution of calories to the metabolic pool even in the absence of an adequate caloric intake.

We also reported studies, using synthetic rations whose dietary nitrogen was supplied entirely by amino acid mixtures, in which we determined the daily essential amino acid "requirements" both for protein repletion and for maintenance. In these experiments the finding of greatest significance was that in every instance the daily utilization rate for a particular essential amino acid in protein repletion exceeded the rate for that particular amino acid for maintenance by a factor of from 2 to 5. On the basis of these results, we suggested that in the process of nutritional rehabilitation of debilitated subjects, estimates of protein needs based on daily essential amino acid requirements as established in normal subjects should be at least doubled for severely depleted patients.

Currently we are using the method for the study of electrolyte needs in tissue synthesis. In two papers we have shown the importance of potassium deficiency in the induction of myocardial necroses and the adverse action of sodium ions in accelerating the development of such lesions. In connection with these studies we also demonstrated the serious degree of inadequacy of potassium in two protein hydrolysates which were otherwise of excellent nutritional quality.

For a number of years the evaluation of the protein adequacy of protein hydrolysates has been a perplexing problem. Part of the difficulty has been due to variations in peptide content of these products and the conflicting points of view concerning the parenteral utilizability of peptides. With the repletion method we have been able to feed depleted rats a nonprotein ration which is adequate in calories, vitamins, salts, and roughage, while at the same time furnishing them dietary nitrogen by multiple subcutaneous injections of hydrolysates containing similar amounts of nitrogen. With 2 hydrolysates so tested protein repletion has been excellent; with 2 others the results have been definitely inferior.

A final word should be said also about the use of the method for the study of amino acid antagonists. In collaboration with Dr. Karl Dittmer, we reported experiments in which B-3-thienylalanine was used as an antagonist for phenylalanine. We found that this substance inhibited weight recovery in protein-depleted rats eating a synthetic ration containing all the essential amino acids in adequate amounts but that "this inhibition was completely prevented by additional amounts of phenylalanine."

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Introductory Studies on Quantitative Relationships Between Tissue Enzymes and Dietary Protein

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In order to build body protein, animals must be supplied with exogenous protein, either as whole protein or as a mixture of amino acids. The most important function of dietary protein is to supply amino acids which the organism is unable to synthesize endogenously. If one of the essential amino acids is omitted from the diet, the animal body presumably becomes unable to synthesize body protein. Because of the dynamic nature of nitrogen metabolism in the body, body proteins are continuously broken down, metabolized, and excreted. If they cannot be rebuilt by taking in the essential amino acids for building body protein, the animal ceases to grow, loses weight, and eventually will die. Under these conditions, it is conceivable that loss of the protein involved mainly in structural functions of the animal would not be so detrimental were it not for the fact that tissue enzymes, which are also proteins, are built from the same essential amino acids that are utilized in building body proteins. Therefore, it is possible that the most serious effects of amino acid deficiencies, or for that matter protein deficiencies in general, arise as a result of the effects upon enzyme systems in the animal body.

Before discussing the evaluation of protein adequacy using enzyme activity methods, I would first like to review briefly some of the groundwork that was laid relating dietary proteins to tissue enzymes before the topic at hand reached its present state. In 1948 Miller (*9*) investigated the effects of inanition on certain liver enzymes in rats. This interesting and important work indicated that fasting caused a loss of liver catalase, alkaline phosphatase, xanthine dehydrogenase, and cathepsin which paralleled or exceeded the loss of liver protein. Potter and Klug (*10*) a year earlier had observed that liver octanoate and succinate oxidases were depressed in rats fed only 6 to 10 percent protein diets. Lightbody and Kleinman (*3*) reported that the liver arginase activity of rats fed a 6 percent milk protein diet was about one-half that of rats fed a 25 percent milk protein diet. Seifter *et al.* (*13*) made the interesting observations that both liver arginase and D-amino ~~and~~ acid oxidase are lost more rapidly than liver nitrogen in rats fed a nonprotein diet. Moreover, the precursors of coenzymes utilized by many other enzyme systems, niacin and riboflavin, were also lost from the liver when animals were fed a nonprotein ration. However, that the loss of enzyme activity during protein starvation is very probably not a result of loss of coenzymes but of the enzyme protein *per se* was pointed out by the latter authors as well as other workers who have studied these phenomena (*8*).

In 1949 it was observed that liver xanthine oxidase in the rat is especially sensitive to subtle changes in dietary protein (*15*). In experiments in which acid hydrolyzed casein plus tryptophane or an amino acid mix simulating casein was fed to adult rats, it was found that liver xanthine oxidase was considerably more active than that of adult rats fed intact casein at the same nitrogen level. These experiments are shown in table 1. Moreover the xanthine oxidase activity could be increased considerably if a little extra methionine was added to the intact casein ration or if the intact casein was raised to 40 percent of the ration. These results indicated that methionine at least was limiting in the 14.6 percent casein ration and that feeding the protein in the prehydrolyzed form made the methionine more available to the rat. Because of the apparent sensitivity of liver xanthine oxidase to dietary methionine, further studies were carried out in which the effects of a complete methionine deficiency were investigated in young rats (*14*). The results of these experiments are presented in table 2. Here it can be seen that the methionine deficiency caused a complete loss in liver xanthine oxidase indicating again the sensitivity of the enzyme to dietary methionine. The other enzyme systems studied decreased somewhat because of the methionine deficiency but not as dramatically as xanthine oxidase.

TABLE 1

THE EFFECTS OF METHIONINE, PROTEIN PREHYDROLYSIS, AND LEVEL OF PROTEIN IN THE RATION ON LIVER XANTHINE OXIDASE ACTIVITY IN THE RAT

Ration fed	Number of rats	Xanthine oxidase activity per gm liver protein
I. 14.6 percent casin	6	590
II. 14.35 percent casein + 0.25 percent dietary methionine	6	1, 080
III. 18 percent acid hydrol casein + 0.5 percent dietary tryptophane	8	900
IV. Amino acid mix at level corresponding to Ration III	6	1, 100
V. 40 percent casein	6	1, 200
VI. Stock	6	1, 200

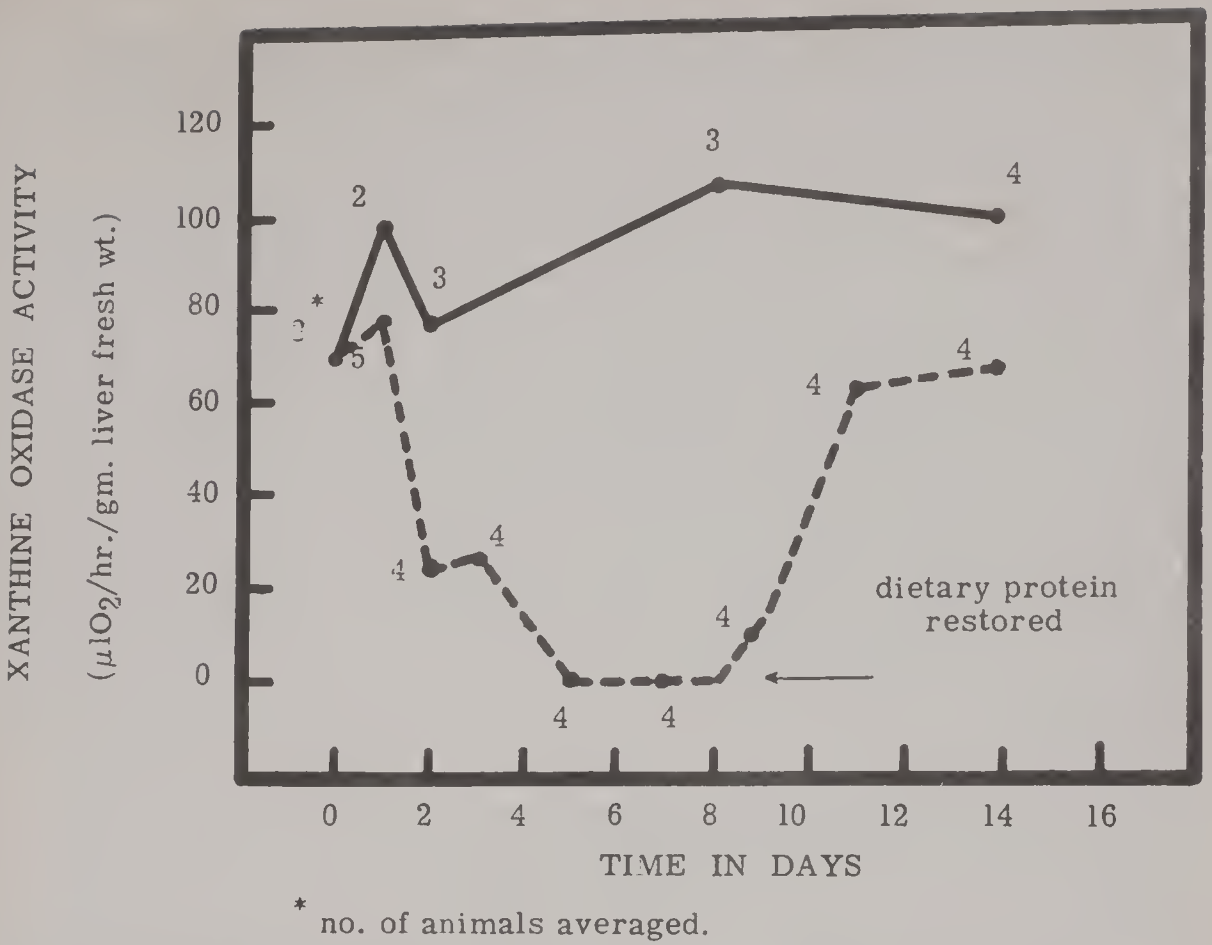

FIGURE 1.—Effect of a nonprotein ration on liver xanthine oxidase activity.

TABLE 2

EFFECT OF METHIONINE DEFICIENCY ON LIVER ENZYME ACTIVITY (FORCE-FED REGIMEN)

Enzyme	Normal	Activity (μlo$_2$/hr./gm. liver) Methionine deficient
Xanthine oxidase	[1] 137 (9)	0 (6)
Succinic oxidase	18, 400 (9)	13, 600 (6)
Endogenous respiration	1, 520 (9)	1, 065 (6)

[1] Figures in parentheses refer to number of animals used for the determination.

Since liver xanthine oxidase is apparently very sensitive to changes in dietary methionine, it would be interesting to see the effects of complete protein deprivation on this enzyme. In these experiments (*8*) adult rats were divided into two groups, one of which received a complete casein ration and the other of which received a nonprotein ration similar in all other respects. Liver xanthine oxidase activity was measured at certain intervals after placing the animals on these diets. The results are shown in figure 1. After 5 days of feeding the nonprotein ration, liver xanthine oxidase activity was completely undemonstrable. When casein was restored to the ration, the enzyme activity increased rapidly. The upper curve shows the normal variation experienced in measuring the activity of this enzyme since that was the group receiving the complete ration throughout the experiment.

Since either a methionine deficiency or complete protein deprivation induces a complete loss in liver xanthine oxidase activity, it might be expected that a deficiency of any essential amino acid would induce a similar loss in xanthine oxidase activity. Two other amino acid deficiencies have been studied in this respect. In table 3 are shown the effects of a histidine deficiency on several liver enzymes (*1*). In marked contrast to a methionine deficiency, xanthine oxidase is apparently unaffected by a histidine deficiency. The slight decrease in succinic oxidase is significant but is not quite as great as with a methionine deficiency. The slight apparent increase in endogenous respiration is not significant because of the large variation encountered in assaying this activity. No change in choline oxidase activity occurred.

TABLE 3

EFFECT OF HISTIDINE DEFICIENCY ON LIVER ENZYME ACTIVITY (FORCE-FED REGIMEN)

Enzyme	Normal	Activity ($\mu l o_2$/hr./gm. liver) Histidine deficient
Xanthine oxidase	[1] 119 (7)	122 (6)
Succinic oxidase	18, 700 (7)	15, 900 (6)
Endogenous respiration	650 (7)	820 (6)
Choline oxidase	2, 480 (7)	2, 570 (6)

[1] Figures in parentheses refer to number of animals used for the determination.

With a lysine deficiency (table 4) [1] liver xanthine oxidase activity is decreased about 50 percent. Succinic oxidase is also quite markedly depressed, while endogenous respiration is only slightly depressed. There is also a decrease in liver choline oxidase with a lysine deficiency.

TABLE 4

EFFECT OF LYSINE DEFICIENCY ON LIVER ENZYME ACTIVITY (FORCE-FED REGIMEN)

Enzyme	Normal	Activity ($\mu l o_2$/hr./gm. liver) Lysine deficient
Xanthine oxidase	[1] 134 (7)	70 (10)
Succinic oxidase	18, 100 (14)	10, 900 (15)
Endogenous respiration	2, 040 (13)	1, 710 (15)
Choline oxidase	1, 490 (10)	1, 000 (13)

[1] Figures in parentheses refer to number of animals used for the determination.

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[1] Unpublished results. In these experiments the methods employed are the same as those used for the methionine and histidine deficiency studies.

What is the significance of the results with amino acid deficiencies? First of all the results indicate that a deficiency of one essential amino acid does not necessarily produce changes in liver enzymes similar to those produced by other essential amino acids. Also in many cases the changes brought about do not appear to be particularly drastic. Nevertheless, after about 2 weeks of force feeding any of the three deficient rations, the animals begin to die. It is possible that in animals fed an amino acid deficient ration, even as in these cases with young growing rats, there are enough body stores of essential amino acids to maintain liver enzymes to some extent provided a normal level of all other essential and nonessential amino acids is fed. From a comparison of the effects of a nonprotein ration (fig. 1) with the effects of a histidine or lysine deficiency on liver xanthine oxidase, it appears, at least in the case of histidine and lysine, that enough stores of these essential amino acids are present in the weanling rat to maintain liver xanthine oxidase until death, provided all other amino acids are given to the rat.

Because of the marked response of liver xanthine oxidase to total dietary protein, a study was made of the relative effects of essential and nonessential amino acids in maintaining activity of this enzyme. In addition, the possible substitution of nonessential amino acids by ammonium nitrogen and urea nitrogen was studied (*6*). In table 5 are presented the results of these studies. Weanling rats were fed the various rations shown in the table for 4 weeks, then sacrificed for the determination of liver xanthine oxidase activity. Weight changes of the rats throughout the feeding period are also presented. From these results it can be seen that neither essential or nonessential amino acids alone, at the levels fed, support good xanthine oxidase activity as well as growth. Ammonium citrate was able to substitute for the nonessential amino acids in both growth and xanthine oxidase activity. Urea was somewhat less active than ammonium citrate for maintaining growth and xanthine oxidase activity although it was definitely still active for both.

TABLE 5

UTILIZATION OF AMINO ACIDS AND NON-AMINO ACID NITROGEN FOR MAINTENANCE OF LIVER XANTHINE OXIDASE ACTIVITY

Ration	Number of rats	Xanthine oxidase activity (μlo/hr./gm. liver)	Average weekly change in weight
Essential amino acids alone	8	48 ± 9	5.1
Nonessential amino acids alone	6	23 ± 15	−2.4
Essential + nonessential amino acids	10	120 ± 12	9.2
Essential amino acids + ammonium citrate	8	98 ± 10	9.9
Essential amino acids + urea	8	87 ± 14	8.6

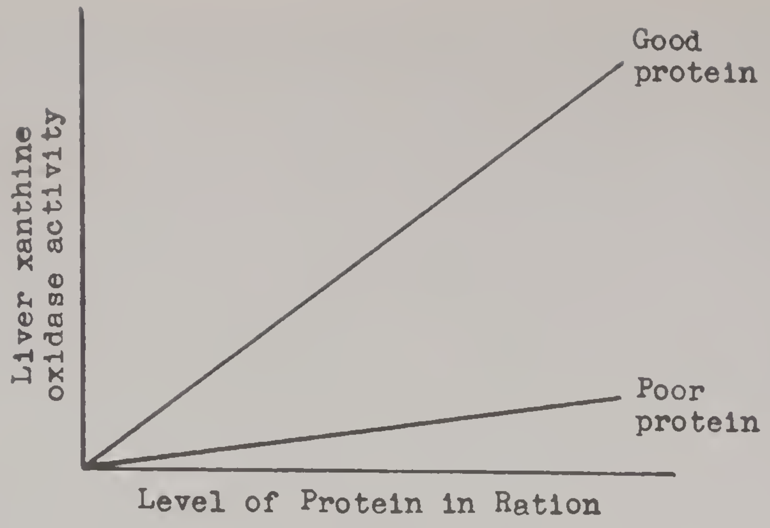

Figure 2.—Hypothetical response of liver xanthine oxidase to a protein of good quality and to a protein of poor quality.

From the results given thus far as well as results from other published information which time does not allow to be presented here, it becomes evident that tissue enzymes respond markedly and quickly to changes in dietary protein. In fact the rapid response of xanthine oxidase in the adult rat to protein deprivation and resupplementation indicated that this effect might be put to some use in studies of the relative values of various dietary proteins. Since xanthine oxidase is a protein itself which responds so sharply to dietary protein changes, the possibility existed that the value of proteins in terms of body protein metabolism could be put into quantitative terms using liver xanthine oxidase as the dependent variable. In casting about for a possible quantitative relationship between liver xanthine oxidase and the value of proteins, it can be imagined (in hypothesis) that if the concentration of protein in a ration is varied, liver xanthine oxidase might respond in proportion to the level of protein in the ration. Moreover it is possible that the response in activity of the enzyme might be different for different proteins, being greater per unit increase in dietary protein of high quality than for a protein of poor quality. Thus as shown in figure 2 we might imagine this situation to be represented by 2 curves, 1 for a protein of good value and the other for a protein of poor value. If this situation actually occurred experimentally, at once we can see a means of relating value of the protein to the response in liver xanthine oxidase activity. By calculating the slope of the two curves, one might obtain a quantitative relationship between liver enzyme activity and the value of the proteins.

In the first actual experiments of this type, casein was chosen as an example of a good protein and gliadin as an example of a poor protein (*5*). When these two proteins were fed to groups of adult rats at different levels in the rations for 10 days and the animals sacrificed for liver xanthine oxidase determinations, the results shown in figure 3 were obtained. It was known that 10 days feeding would be long enough, since in figure 1 it was shown that adult rats reached equilibrium with a nonprotein ration within 5 days, and, also experiments were run to show that the slope of the curves as shown in figure 3 did level off completely by 10 days feeding (7). To correlate the xanthine oxidase response with another criterion of value, weanling rats were fed the same proteins at the same levels for 1 month, and the weight response was plotted in the same way as the xanthine oxidase response. The results of these experiments are presented in figure 4. The similarities of the preceding figure and this one are apparent.

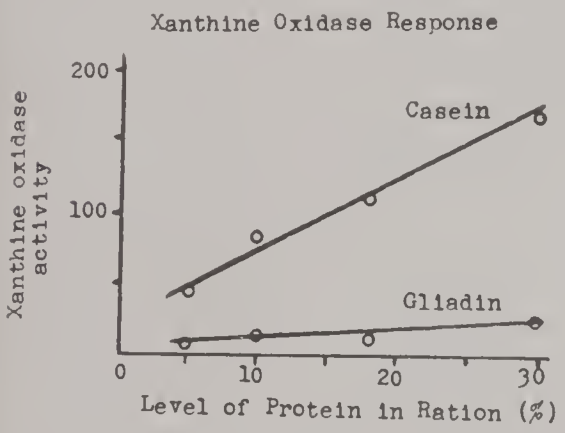

FIGURE 3.—Experimental response of liver xanthine oxidase to casein and gliadin.

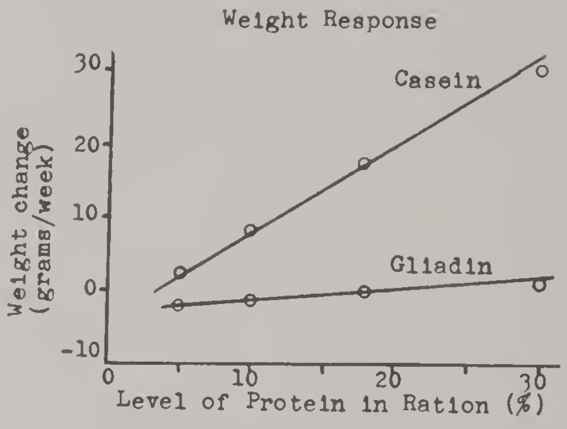

FIGURE 4.—Experimental response of weight of weanling rats to casein and gliadin.

This method of study has been applied to a number of different proteins (7). Also the value of poor proteins after supplementation with amino acids thought to be low or lacking in those proteins has been studied. The results of some of these studies are presented in table 6. The response in xanthine oxidase to dietary protein is expressed as xanthine oxidase activity (in microliters of oxygen uptake

TABLE 6

RESPONSE OF LIVER XANTHINE OXIDASE IN RATS TO VARIOUS DIETARY PROTEINS

Protein studied	Number of rats used in the determination	Xanthine oxidase response (slope of response curve)
Casein	90	5. 7
Lactalbumin	32	5. 0
Beef round	32	7. 0
Gliadin	66	. 8
Gliadin + tryptophane	26	1. 2
Gliadin + lysine	16	4. 0
Gliadin + tryptophane lysine	12	5. 1
Gliadin + amino acids to simulate casein	14	4. 2
Hydrolized gliadin	13	. 6

per hour per gram liver) per percent of dietary protein. The relatively large numbers of animals employed for the first four proteins in this table were used to establish firm values for these fairly representative proteins. Of the list, beef round appears to give the best xanthine oxidase response followed by casein and lactalbumin, with gliadin running a very poor fourth. Attempts to increase the value of gliadin by supplementing it with amino acids known to be low were somewhat successful. The addition of lysine gave a much greater response. When both lysine and tryptophane were added the value of gliadin was brought almost to that of casein, but not quite. When other amino acids were added to gliadin in addition to lysine and tryptophane, there was a slight recession in response which cannot yet be explained. Finally it was thought that, by hydrolyzing gliadin to its free amino acids, its value might be increased. This would occur if one reason for the low value of gliadin were its poor digestibility. However, prehydrolysis did not improve its value. In order to observe whether a correlation between the xanthine oxidase response constant and growth exists, weanling rats were fed the proteins listed in table 6 (except for beef round) at the various levels employed for determination of the xanthine oxidase response constant. After 1 month of feeding, the change in weight for each group at each protein level was plotted versus percent of protein in the diet. The slopes of the resulting curves were then calculated by the method of least squares as in determining the xanthine oxidase response constant. The ratio of the xanthine oxidase response constant (slope) to the weight response constant (slope) was then calculated, and the results are presented in table 7. The variation in these ratios indicates that the correlation betwen the xanthine oxidase and weight responses is by no means perfect. However, the fact that they are fairly close together indicates that a good or poor weight response can be predicted by the xanthine oxidase response. Probably a perfect correlation between growth and xanthine oxidase response should not be expected since weight increase

TABLE 7

CORRELATION BETWEEN THE XANTHINE OXIDASE RESPONSE CONSTANT IN ADULT RATS AND THE WEIGHT RESPONSE CONSTANT IN WEANLING RATS FED VARIOUS PROTEINS

Protein studies	Ratio of xanthine oxidase response (adult rats) to weight response (weanling rats)
Casein	2.8
Lactalbumin	2.9
Gliadin	2.1
Gliadin + tryptophane	2.4
Gliadin + lysine	2.0
Gliadin + lysine	2.8
Gliadin + lysine	

is the culmination of a large number of factors in addition to protein intake, such as water intake and balance, fat deposition, and hormonal state of the animal. Since xanthine oxidase is apparently one of the labile proteins of animal tissues, its response should reflect protein synthesis or loss in the body. Since it responds so rapidly to changes in exogenous proteins, and since it is a particular body protein which can be easily measured, it should be a fairly suitable criterion for estimating the value of dietary protein in synthesizing labile body protein.

Dietary molybdate has been shown to influence markedly the level of xanthine oxidase in rat intestine (*2*, *11*). Whether this ion has any effect on *liver* xanthine oxidase is a question that has to be answered before the liver enzyme can be used to measure protein adequacy. Richert and Westerfeld (*12*) recently studied this question and concluded that the level of liver xanthine oxidase depends primarily on the protein content of the diet and that molybdate has no demonstrable effect under the conditions of their experiments. The same question has been studied using the methods employed for determining the xanthine oxidase response constant as reported in the preceding discussion (*4*). In these studies sodium molybdate was added to the protein to be studied at a level of 0.5 mg. percent. The combination of protein and molybdate was then assayed by the xanthine oxidase method at 5, 10, 15, and 20 percent levels of the protein in the diet. A comparison with the same protein lacking added molybdate was also made. The results of these studies with lactalbumin and casein as test proteins indicated that the same response constants were obtained whether molybdate was present or absent from the ration. Therefore, the question of molybdate interference in the xanthine oxidase assay for protein adequacy can probably be ruled out.

Thus far all the work concerning the xanthine oxidase response constant (except for the molybdate studies) has been carried out using adult rats as test animals. Whether weanling rats can adequately be used as test animals is a question that can only be answered by experimental work. At weaning, rats have very low liver xanthine oxidase activity which gradually increases with age. This fact, therefore, complicates the use of weanling rats as test animals. However, some experiments have been carried out using weanling rats in the xanthine oxidase test (*4*). In these studies weanling rats were fed various levels of the protein in question for 21 days before sacrifice for xanthine oxidase determinations. Weight records were also kept for these animals. The results of these experiments are presented in table 8. Here it can be seen that the enzyme response constants for casein and lactalbumin are lower than those obtained with adult rats while gliadin gives a higher enzyme response than that obtained with adult rats. The correlation with growth, although good from a directional point of view, is not as good on a numerical basis as with adult rats.

TABLE 8

RESPONSE OF LIVER XANTHINE OXIDASE AND GROWTH IN WEANLING RATS TO VARIOUS PROTEIN PREPARATIONS

Protein studied	Number of rats used for the determination	Xanthine oxidase response constant (A)	Weight response constant (B)	Ratio of A:B
Casein	56	3. 5	1. 6	2. 2
Lactalbumin	24	3. 9	1. 8	2. 2
Gliadin	48	1. 5	. 06	25
Corn-soya	32	3. 5	1. 7	2. 1

Thus far, the results presented relating dietary protein to liver enzyme activity are entirely preliminary and many more factors must be studied before the real meaning of these results becomes established. Some of the factors that should be studied are the effect of the enzyme assay temperature on the response constant, variation in the response constant from one sample of protein preparation to another, whether the method of calculation is sound (i. e., whether the assumed straight line relationship between xanthine oxidase activity and percent of dietary protein holds up under many sets of conditions), and further elaboration of the relationship between the xanthine oxidase response, growth, and protein utilization as measured by other methods. Thus we can see that the studies presented in this discussion are still in a preliminary stage, but they hold promise of offering a specific and easily performed method for the evaluation of dietary proteins.

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Other Methods of Evaluation

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The determination of the nutritive value of a dietary protein involves measurements of the ability of the diet to promote growth, maintenance, and the general welfare of each tissue within the animal. Early studies on nutritive value placed emphasis upon growth of the whole animal, growth being considered as one of the best single measurements of tissue welfare.

Growth of young animals. Most of the procedures involving growth have been adaptations of the growth response test devised by Osborne, Mendel, and Ferry (*24*). These adaptations included the addition of protein to some standard ration at a concentration (usually 10 percent) that was known to be insufficient for optimal growth. Food intake and rate of growth were measured in terms of the protein efficiency ratio (grams gain in weight per gram of nitrogen intake). Diets were usually fed *ad libitum*, and experimental animals, in general, increased their food intake as the nutritive quality of the protein in the diet increased. Under standardized conditions the gain in weight was correlated nicely with nitrogen retention in the carcass (*13*). Indeed, Hegsted and Worcester (*20*) found a very high correlation between gain in weight and protein efficiency measurements in rats, a correlation which suggested that little additional information was gained by calculating efficiencies. It might be stated that a high correlation between gain in weight and nitrogen retention could be one criterion for "normal" growth in young animals.

Recently, cooperating laboratories (*16*) determined gain in weight and protein efficiencies in rats while feeding different standardized protein sources. The results of these studies are summarized in table 1 where protein efficiencies and increases in body weight are expressed

TABLE 1

PROTEIN EFFICIENCIES AND INCREASE IN WEIGHT DETERMINED IN RATS, THE RESULTS BEING EXPRESSED AS PERCENT OF THE VALUES OBTAINED FOR WHOLE EGG

(Average data from seven laboratories (*16*))

	Whole egg	Egg white	Beef muscle	Casein	Peanut flour	Wheat gluten
Protein efficiency	100	107	89	90	46	14
Weight gain	100	105	86	84	32	8

as percent of the value obtained for whole egg. The significance of protein efficiency ratios as a measure of nutritive value can be expressed as follows: Egg white $\underset{>}{s}$ whole egg $\underset{>}{hs}$ beef $\underset{=}{ns}$ casein $\underset{>}{hs}$ peanut flour $\underset{>}{hs}$ wheat gluten. (NS=not significant, S=significant, HS=highly significant.) These results support the confidence commonly placed in this method of estimating nutritive value. Relatively similar average values for increases in body weight are also recorded. There was, however, considerable more variation in weight gains than in protein efficiency ratios, the coefficient of variation with body weight increase being greater than twice that with the ratios. These results suggest that nutritive values can be more easily uncovered through ratios than through increase in body weight (*7*).

The determination of protein efficiency, however, must be standardized so that there is known to be a high correlation between gain in weight and nitrogen retention, otherwise the results can be very misleading. The possibility of low correlation has been pointed out by Mitchell (*22*) who has discussed thoroughly the shortcomings of this method of protein evaluation. Most of the shortcomings are functions of the effect of the diet and food intake on the protein, fat, and water content of tissues. An agar gel type of diet has been developed in our laboratories (*5*) which is eaten well, especially by dogs, even when fed free of protein or when containing a protein of poor quality such as wheat gluten. One group of puppies, fed *ad libitum* this diet containing wheat gluten, had a higher caloric intake than a similar group fed the same diet containing whole egg (*3*). The puppies eating the diet containing wheat gluten were fat while those eating the diet with whole egg were not. The gain in weight per gram of nitrogen intake (protein efficiency) was similar in both groups but the nitrogen retained was much greater in animals fed the whole egg than in those fed wheat gluten.

This lack of correlation between gain in weight and nitrogen retention can be associated in part with variation in caloric intake, a factor which has been analyzed recently by Spector and Calloway (*9*, *26*). Even under conditions of paired feeding, gain in weight is not always correlated highly with nitrogen retention. Beadles *et al.* (*8*), for example, demonstrated in paired-feeding tests that the protein of Limburger cheese was equal to fresh milk curd in promoting increase in weight but the gain in animals fed the cheese ration was due less to protein and more to fat than in animals fed the curd.

Barnes and Bosshardt (*7*) have pointed out that paired feeding may give artificial protein efficiency ratios, particularly if the animals are pair fed to a group receiving a protein of poor quality where food intake is restricted. If restriction is severe, some of the better protein may be used to correct for the caloric deficiency. These authors

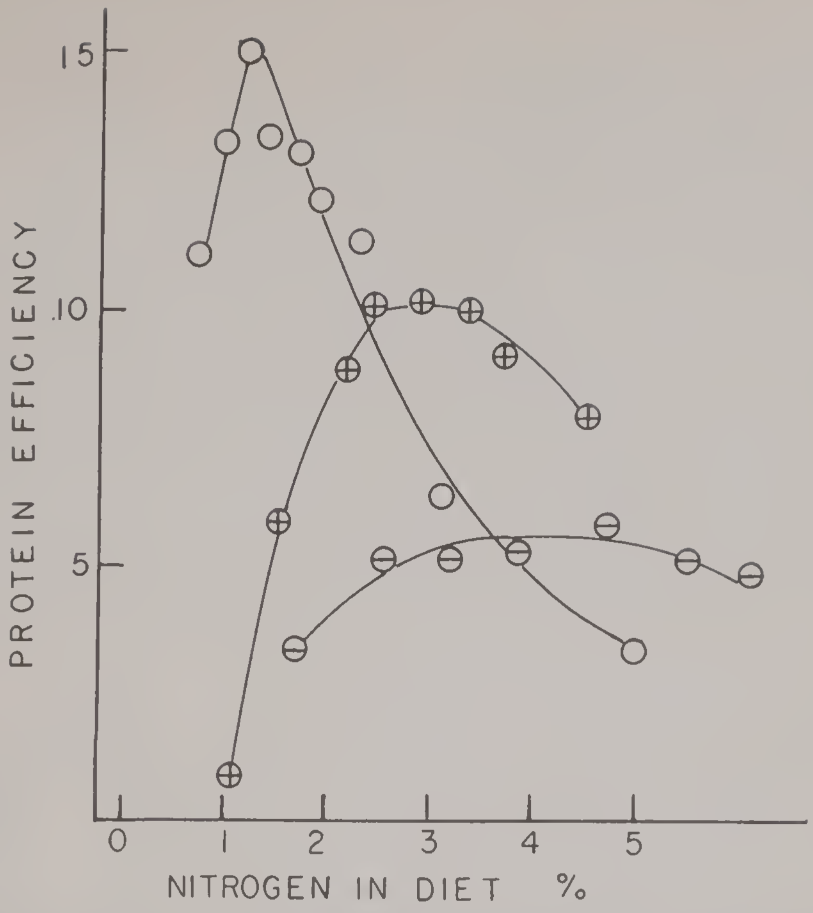

FIGURE 1.—The variation in protein efficiency ratios with nitrogen content of the diet, data obtained by Barnes and Bosshardt (*16*) while feeding mice the following protein sources, whole egg (open circles), peanut flour (circles with crossed bars), wheat gluten (circles with horizontal bars).

emphasize that protein efficiencies vary with nitrogen intake, rising to a maximum and then decreasing to lower values as the intake increases. Indeed Osborne, Mendel, and their associates used the maximum ratio as the index of nutritive value for growth. Barnes and Bosshardt (*7*) present data to demonstrate that some of the shortcomings of the method can be overcome if this original longer procedure is followed. The data, illustrated in figure 1, were obtained by Barnes and Bosshardt (*16*) while feeding mice different amounts of nitrogen in the diet. These data illustrate the necessity for selecting carefully the percent nitrogen in the diet if one nitrogen content is used to compare efficiency ratios for various proteins. The ratio for whole egg protein, for example, is equal to the ration for wheat gluten when the nitrogen content of the diet is approximately 3.5 percent but the ratio for whole egg protein is higher than the others when the dietary nitrogen is reduced to 2 percent. It is possible that the difference in the shapes of these curves also has significance, reflecting variations in rates of filling different tissue protein stores.

These data and others demonstrate that protein efficiencies and weight gains can be correlated highly with some function of nutritive value of proteins, if the experimental design permits it, but they also emphasize the possibilities of poor correlation under various dietary experiences. These are tests, however, for growth, and nutritive values obtained in this way may not parallel those determined for other functions. Mitchell has shown in the rat, for example, that wheat gluten is relatively adequate for maintenance but quite poor for growth. To quote from Mitchell (*23*), "the amino acid requirements of the growing animal are determined in the last analysis by the amino acid composition of the tissue protein formed during growth." He points out that both increases and maintenance of protoplasmic tissue dominates the requirements for the young while development of hair, wool, feathers, etc., can be an important influence on the requirements of the adult animal. Since the muscle proteins of various animals are similar and represent the greatest potential storehouse of proteins, it is not surprising to find that methods of evaluating dietary proteins involving growth or repletion of the protein-depleted body yield relatively similar values in most animals.

Depletion and repletion of body nitrogen.—Repletion of depleted tissue is another measure of nutritive value, a measure that can be expressed broadly in terms of overall changes in body weight or body nitrogen. Cannon and his associates (*11*, *12*, *17*) have developed the rat repletion method into an outstanding technique for protein assay. This method is being reported by Dr. Cannon so that only a few remarks will be made toward the use of repletion in body nitrogen in the dog as a measure of nutritive value. There is a basic tissue catabolism that results in a continual breakdown of tissue protein stores and loss of nitrogen from the body. Unless this loss is replaced daily by a pattern of dietary amino acids, the protein stores are reduced. The urinary nitrogen excretion is high when the protein stores and catabolic activity are high; the excretion is low when the stores and metabolic activity are low. The open circles in figure 2 illustrate the gradual increase in nitrogen balance, reflecting the decrease in urinary nitrogen in dogs fed a protein-free diet. This value tends to become asymptotic to a nitrogen balance near 1 gm./day/sq. m. of body surface area (*1*). After 64 days on the protein-free diet, the dogs were fed casein equivalent to 0.35 gm. nitrogen/day/kg. body weight. The circles with crossed bars illustrate the effect of feeding the casein over a period of 30 days. Initially the dogs were placed far in the region of positive balance but as the protein stores were filled the balance decreased toward nitrogen equilibrium. Continued feeding of casein would eventually have filled the stores and increased catabolic activity to the point where a relatively large amount of nitrogen would have been needed to maintain equilibrium. Before this degree of repletion was reached, however, the animals were replaced on the protein-free diet. The open circle at 101 days indicates the tendency for catabolic activity to return to the initial higher value.

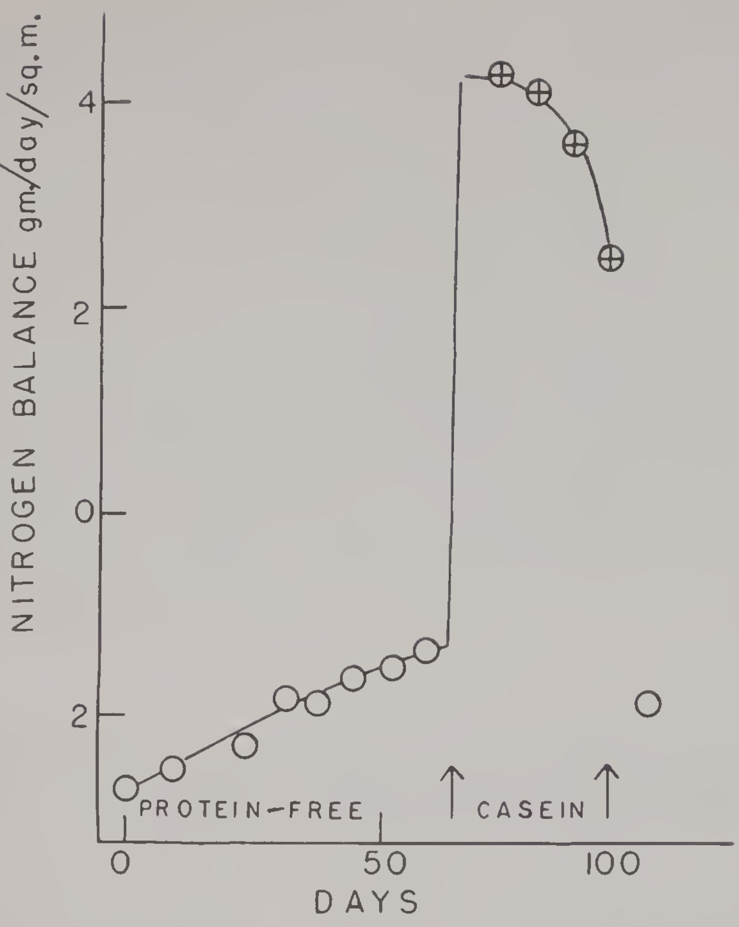

FIGURE 2.—Average nitrogen balances in three adult dogs fed a protein-free diet for a period of 64 days (open circles) followed by a diet containing casein (0.35 gm. nitrogen/day/kg. body weight) for 30 days (circles with crossed bars). The last point at 101 days (open circles) was obtained by feeding a protein-free diet for several days following the period of repletion.

A poor-quality protein, such as wheat gluten, does not place the animals so far in positive balance and does not show so rapid a drop toward equilibrium. The amount of nitrogen balance produced during repletion is another measure of nutritive value of the protein. In one experiment it was estimated that with a constant intake of nitrogen the following total positive balances were produced in dogs over a period of 30 days of repletion: whole egg 105, casein 100, and wheat gluten 45. There was no significant difference between whole egg and casein but wheat gluten was markedly lower than the other two (*4*).

This method of comparing nutritive value of proteins is tedious but has the advantage, however, of measuring the ability of the diet to repair the damage resulting from protein starvation. Nitrogen balances can be misleading, however, since they may be the algebraic sums of gains and losses. Two proteins producing the same nitrogen balance can fill the protein stores of the animal differently (*1*). A somewhat simpler and more direct approach to the study of the effect of dietary protein on loss and gain of protein stores has been to measure changes that take place in a specific tissue. It should be emphasized at the outset, however, that the amino acid requirement for repletion of any tissue protein can vary according to the structure of that protein, and the position it holds in the dynamic state of the body so that nutritive values determined through repletion of specific tissue proteins need not parallel those estimated in other ways.

Depletion and repletion of plasma proteins.—Maintenance of so-called normal plasma protein concentration has been used to determine the effect of diet upon the welfare of protein stores of the animal. This method of estimating the magnitude of the protein stores is limited, in part, because protein concentration does not always reflect accurately total circulating protein. In general, as a dog is depleted the plasma protein concentration decreases from values around 6 to values as low as 4 gm. percent. There are many exceptions to this decrease, however, exceptions which are associated with changes in blood volume. Thus an animal can be markedly depleted in proteins but still have a normal plasma protein concentration, the fall in total circulating proteins being paralleled by a loss in fluid (*2*).

Depletion in plasma proteins means loss in plasma albumin (*15*). This loss is illustrated by data summarized in table 2, data obtained on one of the dogs depleted and repleted in the experiment illustrated in figure 2. The percent of plasma albumin was reduced during 64 days on a protein-free diet while the α globulins increased. The net result was a marked drop in the A/G ratio. Thirty days of repletion on the casein diet did not return the plasma albumin to normal, a result which is in agreement with the overall measurements of nitrogen retention illustrated in figure 2.

TABLE 2

The Electrophoretic Pattern of Plasma Proteins in a Dog Fed a Protein-Free Diet for 64 Days Followed by Repletion With Casein for 30 Days

(0.35 gm. casein nitrogen/day/kg. body weight)

Days	Diet	Albumin (A)	Globulins (G)				
			α1	α2	β	γ	A/G
		Percent					
0	Protein-free	38. 3	3. 6	6. 6	33. 2	18. 4	0. 62
28	Protein-free	25. 2	9. 3	14. 8	19. 6	19. 7	. 30
64	Protein-free	14. 2	11. 5	23. 0	29. 5	30. 1	. 17
30	Casein	25. 9	14. 5	19. 9	13. 8	25. 9	. 35

The decrease and increase in A/G ratio reflects the depletion and repletion in protein stores and has been used as a measure of nutritive value, dietary proteins repleting the ratio toward normal. Casein, however, tends to increase globulins above normal during repletion so that the A/G ratio is low in animals repleted with casein (*14*). It is interesting to reflect that hypothyroidism, no matter how induced, also increases the globulin fraction, thereby reducing the ratio.

Even in growth of young animals the gain in weight can at times be correlated with the albumin fraction of the plasma. The data in table 3 illustrates the greater gain in weight and the greater percentage of plasma albumin in rats fed casein supplemented with methionine than in control animals fed casein alone. An animal can be badly depleted, however, and have a high A/G ratio. A growing tumor, for example, can deplete the soft tissues of the body in proteins, even reduce the plasma protein concentration to low values and yet the percent of albumin may remain normal. Anomalies such as this can be explained by assuming that the tumor, growing at the expense of the other tissues of the body, keeps the transporting proteins of the blood near normal of equilibrium.

TABLE 3

ELECTROPHORETIC PATTERNS OF PLASMA PROTEINS OBTAINED FROM NORMAL RATS GROWN FOR 37 DAYS ON A DIET CONTAINING 12 PERCENT CASEIN AND ON THE SAME DIET SUPPLEMENTED WITH 0.67 PERCENT METHIONINE

Diet	Increase in body weight gm.	Plasma protein gm. percent	Albumin (A)	Globulins (G)				
				$\propto 1$	$\propto 2$	β	γ	A/G
				Percent				
12 percent casein	112	5.8	36.8	24.0	12.8	19.0	7.6	0.58
+0.67 percent methionine	147	6.9	45.8	20.2	13.7	15.0	5.2	.84

Whipple, Robscheit-Robbins (*25*), and their associates have done much to determine the nutritive value of proteins for the purpose of forming blood proteins. In their most recent technique they have doubly depleted dogs by feeding a protein-free diet plus frequent blood removals over a period of 3 to 4 weeks. The plasma proteins were reduced to approximately 4.8 gm. percent and the hemoglobin to 6.1 gm. percent. Hemoglobin and plasma protein outputs per week were determined during repletion as grams removed by bleeding. They found that some diets favored the formation of plasma proteins, others favored production of hemoglobin, some promoting both equally well. Their results re-emphasize the fact that amino acid requirements for repletion of specific tissue proteins vary according to the structure of that protein and the position it holds in the dynamic state of the body.

Tissue proteins.—Other tissues of the body are depleted in labile proteins when animals experience periods of fasting or diets low in calories or proteins. Not all tissues, however, are depleted in the same way. The data in table 4, for example, illustrate the effect on liver or heart proteins of feeding a protein-free diet to rats. Depletion reduced total protein of both organs but the percentage of protein (dry weight) was not reduced in the heart but was reduced in the liver. This difference in the type of depletion in these two organs is reflected also by changes in activities of enzyme systems. The unit activities (per mg. nitrogen) of cytochrome oxidase, succinic dehydrogenase, and DPN-cytochrome *c* reductase were not altered by depletion in the heart but in the depleted liver, cytochrome oxidase was increased to approximately 130 percent of normal while succinic dehydrogenase and DPN-cytochrome *c* reductase were reduced to less than 50 percent of normal. Thus an imbalance in the activities of these and other enzyme systems developed in the liver, an imbalance which was not observed in the heart (*27*).

TABLE 4

Effect of Depletion in Proteins on Liver and Heart in Rats (*4*)

	Body weight gm.	Organ weight gm.	Protein	
			gm.	Percent dry weight
			Liver	
Control	462	14. 54	2. 4	58. 4
Depleted	202	11. 36	1. 2	31. 7
			Heart	
Control	462	1. 26	0. 17	70. 1
Depleted	202	. 64	. 10	71. 3

Liver protein, like plasma protein, has been used to estimate the nutritive value of dietary proteins. Campbell and Kosterlitz (*10*) laid the foundation for this estimation when they reported that the amount of labile cytoplasm present in the liver is directly proportional to the logarithm of the protein intake. They developed two methods for the assay of proteins, one measured the ability of dietary protein to maintain liver cytoplasm when the animals were transferred from a stock diet to a test diet, the other measured the ability of the protein to increase labile cytoplasm after the rats had been on a protein-free diet for several days. Similarly, Harrison and Long (*19*) assayed protein adequacy by determining the regeneration of liver protein in rats depleted under standard conditions. They found, for example, that regeneration of liver proteins by dietary proteins could be arranged as follows: lactalbumin=casein>gliadin>zein>gelatin. Gurd, Vars, and Ravdin (*18*) studied the formation of new liver protein following hepatectomy. They concluded that "a close correlation appeared to exist between the amount of new liver protein formed and the amount of nitrogen saved or spared, irrespective of whether the rats were in positive or negative nitrogen balance. It is suggested that any factor, or summation of factors which causes an overall change in nitrogen metabolism will affect the liver protein." These data and others support the belief that variation in nitrogen balance is reflected quantitatively by changes in liver protein.

Knowledge, however, of the physiological state and variations associated with that state are essential to interpretation of the data obtained. Hyperthyroidism, for example, tends to increase liver protein even though the animal may be in negative nitrogen balance (*21*). Indeed it is possible that the increased catabolism associated with hyperthyroidism may bring to the liver larger quantities of liver protein building materials preventing the depletion of that organ. A similar situation developed in rats with transplanted cancer, rats which were severely depleted in nitrogen by the tumor but with relatively high liver proteins (table 5).

TABLE 5

LIVER PROTEIN IN RATS WITH 18-DAY TRANSPLANTED SARCOMA AND IN PAIR-FED CONTROLS (*6*)

Diet	18 percent casein	9 percent casein	Protein free
Liver protein controls mg./100 gm. body weight	659	557	562
Tumor animals mg./100 gm. carcass	853	813	658

Reproduction and lactation.—Data have been presented to show that depletion in tissue proteins is not uniform, some proteins being depleted more than others, some tissues more than others. It has been our experience that the tissues involved directly in reproduction in the rat are resistant to depletion. Badly protein-depleted rats, given all other nutritional factors associated with reproduction, can still reproduce, although the growth of the young during lactation will vary with the nutritional value of the protein in the diet. Seeley (*16*) found, for example, that the weanling weights at 21 days of rats varied according to the diet fed to the mothers as follows: whole egg=beef muscle>casein>peanut flour>wheat gluten.

Nutritive value and function measured.—It seems appropriate to end this survey of several methods for evaluating dietary proteins by emphasizing once more than the results obtained will depend upon the experimental design, and upon the function that is being measured. Nutritive values may be established for growth, for maintenance, for repletion of the body as a whole, or for specific tissues, or proteins within those tissues. These values may vary according to the function measured. In general, however, nutritive values reported in the literature agree quite well because the body is an integrated mechanism and the measurements made were functions of that integration.

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III. Evaluation of Vitamin Adequacy

THURSDAY AFTERNOON SESSION

25 February 1954

Blood Levels

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The use of blood levels of the vitamins or of products related to their metabolic action or deficiency as a means of evaluating vitamin adequacy requires: (*a*) practicable analytical methods for measuring the concentrations of the various vitamins or their direct or indirect metabolic products in blood; (*b*) satisfactory methods for measuring the physiological status of the subject over a wide range of vitamin intake; (*c*) a knowledge of the relationship between the two variables, blood level and physiological status, over a wide range of intake; (*d*) knowledge of the factors other than intake that may influence blood levels and of the factors other than intake that may influence requirements; and, finally, (*e*) there must be an understanding of the criteria of adequacy that are being used in a particular situation, in order to avoid misinterpretation of the analytical data. This sounds like a complicated picture, and it is. Although there is considerable work yet to be done and much understanding of the proper use of such methods yet to be developed before these methods will reach their ultimate usefulness, their proper application and interpretation are more useful at the present time than is generally realized.

There is a general tendency to expect more from biochemical measurements of all types than the intrinsic nature of most biological problems will allow. I suspect this is often true when one can express a measurement in figures rather than in judgment. The use of blood analytical methods for the evaluation of vitamin adequacy has been no exception to this tendency. Unfortunately, during their development in the last few years, these methods have been applied in certain instances with disappointing results. Invariably, this has been the result of the use of the wrong tool for the situation, or an unskilled use of the right tool. Now, after several years' experience and time to reflect and evaluate that experience, it seems to me that we have reached a stage in our knowledge of this aspect of the field of nutrition at which we can fairly clearly see the true usefulness as well as the limitation of blood methods as a means of evaluating nutritive status. The discussion that follows is the author's judgment of this situation. No attempt has been made to give a complete literature coverage, but rather references are cited as examples. Analytical techniques will not be dealt with since these are available in detail elsewhere (*25*). However, it can be said that good practicable blood analytical methods are available for most of the vitamins, and while there is room for improvement in this aspect of the problem, it is not a limiting factor at present. Attention will be directed primarily to the problem of the relationship between blood levels and vitamin intakes and how these may be related in an approximate way to physiological status. A discussion of the more exact relationship of intake to physiological status, and the problems concerning criteria of adequacy would require more time than there is available.

Ascorbic Acid

Plasma or whole blood.—The fasting blood plasma level of a well-nourished adult (70 to 100 mg. ascorbic acid intake daily) decreases from 0.6–1.0 mg./100 ml. to 0–0.1 mg./100 ml. in 10 to 14 days when he is placed on an ascorbic acid-free diet (*9*, *23*). Continuation on such a regimen will lead to the appearance of clinical evidence of scurvy in 4 to 5 months. Plasma levels remain below 0.1 mg./100 ml. on a 10 mg. daily intake, and below 0.2 mg./100 ml. on a 23 mg. daily intake (*16*, *17*, *23*). Although frank scurvy may not appear under these conditions, indications of gum changes may become evident (*16*, *23*). Ascorbic acid intakes of 25 to 70 mg. daily lead to plasma levels of 0.2–0.6 mg./100 ml. At levels of ascorbic acid intake above 70 mg. daily, blood plasma levels usually vary between 0.6 and 1.0 mg./100 ml. (*3*, *4*, *17*, *20*). These levels may rise to as much as 1.4 mg./100 ml. at intakes of 100 to 1,000 mg. daily (*18*). However, in general, the kidney excretes most of these larger doses and consequently the blood level does not rise farther. There is some evidence that levels of ascorbic acid up to the 0.6–1.0 mg./100 ml. plasma level confer benefits to the gums.

Whole blood values are not significantly different from plasma values (*19*). However, whole blood ascorbic acid must be measured by use of the dinitrophenyl-hydrazine method, or with special precautions to avoid its oxidation during analysis if the indophenol method is used.

White blood cells.—The white blood cell ascorbic acid content of a well-nourished adult (70 to 100 mg. ascorbic acid intake daily) decreases from 20–30 mg./100 ml. to 0–2 mg./100 ml. in 3 to 5 months when he is placed on an ascorbic acid-free diet (*9*, *23*). White blood cell values of 2 to 12 mg./100 ml. are found when the ascorbic acid intake is 10 to 23 mg. per day. Intakes of 23 to 70 mg. daily lead to a gradually increased white cell value up to 20–30 mg./100 ml. (*7*, *17*, *19*, *23*, *26*). This level is not appreciably increased at higher intakes.

Since between individuals whose ascorbic acid intakes are the same there may be considerable variation in both plasma and white blood cell levels of ascorbic acid from individual to individual, the interpretation of these analyses is more reliable when applied to group evaluations where statistical methods are applicable.

Conclusions

1. Plasma ascorbic acid levels below 0.1 mg./100 ml. and white blood cell values below 2 mg./100 ml. should be considered presumptive evidence of incipient scurvy unless additional evidence indicates otherwise. Since these analytical methods become less certain at low concentrations, a plasma value below 0.2 mg./100 ml. would probably be a more reasonable figure for this purpose. Since white blood cell ascorbic acid concentration remains up for a longer time during ascorbic acid restriction and may be normal in certain individuals even when the plasma values are low, such analyses are indicated when serum levels are found low. This allows an estimation of status between intake levels of 0 to 25 mg. ascorbic acid daily, a region in which the sensitivity of plasma analyses is greatly limited.
2. Plasma ascorbic levels above 0.6 mg./100 ml. quite certainly indicate that the dietary intake is 70 mg. or more and that adequacy of the diet in this respect cannot be seriously doubted.
3. Plasma ascorbic acid levels between 0.2 and 0.6 mg./100 ml. are less informative than those at the extremes of the range. However, in general, they mean that scurvy is not imminent and the dietary intake of ascorbic acid is likely somewhere between 25 and 70 mg. daily. Whether or not such levels are adequate depends upon the interpretation of the term "adequacy."
4. Plasma ascorbic acid analyses are reliable and useful in determining and comparing the ascorbic acid intakes of population groups—a situation in which statistical analysis of the data can remove a considerable part of the uncertainty inherent in individual determinations and interpretation (*3*, *8*). This is especially true of low analytical values. It should be reemphasized that a low individual plasma ascorbic acid level does not *prove* the presence or even potential development of a disturbing deficiency. It does, however, indicate a real possibility that should be seriously considered. In any event, it represents a situation that can be changed from a doubtful status by increasing the ascorbic intake by the most practicable means available.

Vitamin A and Carotene

Vitamin A—plasma.—The vitamin A plasma concentrations of a large group of well-nourished children or adults (3,000 or more

I. U. vitamin A daily) will lie, for the most part, between 30 and 50 μg./100 ml. (*3*, *8*). There will be a few in such a group that have values of 20 to 30 μg./100 ml., and a few above 50 μg. When well-nourished adults are placed on a vitamin A-free regimen, their plasma vitamin A levels remain relatively constant for a few months and then slowly decrease over a period of the next several months to levels which may reach 10 μg./100 ml. within a year (*22*). At levels below 15–20 μg./100 ml., evidence of deficiency generally appears (decreased night vision).

Experimental animals receiving graduated intakes of vitamin A show a predictable correlation between intake and plasma level up to 25–30 μg./100 ml., at which point the average vitamin A plasma concentration remains relatively constant with further increased intake, although day-to-day values may show considerable variation (*1*, *11*, *14*, *15*). Very large intakes will increase the plasma levels, but there is a tendency for the latter to remain around the 25–30 μg./100 ml. value. Liver storage of vitamin A in the rat becomes apparent at about this same level. There is evidence that night vision becomes defective in a number of species at vitamin intakes that would allow the blood concentration to fall below 15–20 μg./100. It seems apparent that at vitamin **A** intake levels which lead to liver storage, factors other than intake become important in governing blood levels.

Carotene—plasma.—The plasma carotene concentration in a large group of well-nourished individuals will lie, for the most part, between 75 and 200 μg./100 ml. (*3*, *8*). However, since this depends primarily upon the green and yellow vegetable content of the diet, it may vary greatly within this range from one individual to the next. When adults are placed on a carotene-free diet, the blood level drops rather promptly and may become as low as 20–30 μg./100 ml. within a month or two (*22*).

Conclusions

1. Plasma vitamin A levels below 15–20 μg./100 ml. are presumptive evidence of a deficiency that may be clinically recognizable.
2. Plasma vitamin A levels above 30–40 μg./100 ml. can be taken as evidence that there is storage of this vitamin in the liver.
3. Although there is a rough correlation between blood levels above 20 μg./100 ml. and intake or liver storage, blood levels in this range are subject to change by factors other than intake and are, therefore, not very reliable as an index of intake.

In this case, as with ascorbic acid, these methods are more reliably interpretable when used with groups. As with ascorbic acid, there are a few individuals who, for reasons not yet explainable, will have low vitamin A or carotene blood levels even on a habitual intake generally considered adequate. Likewise, there is evidence that pathological processes such as trauma, certain infections, etc., may lead to low blood values in the presence of what is generally considered an adequate intake. Whether such low levels should be interpreted as an indication of a deficiency is not yet clear and requires further investigation. Perhaps it should be added that the interpretation of blood vitamin analyses in general in pathological situations must be done with caution and with consideration of factors other than intake. The need for such considerations is no more nor less important than for other types of biochemical tests. Even such a well-established analysis as blood sugar can be misleading if not properly applied and interpreted.

4. Low carotene values do not mean vitamin A deficiency, but if found in a population or individual who must depend upon this provitamin A as their chief source of the vitamin, it is informative.

Riboflavin

Table 1 summarizes the results obtained by blood analyses on 6 adult subjects who had been maintained on a controlled riboflavin intake of 2.55 mg./day for several months and 10 similar subjects who received 0.55 mg. of riboflavin/day under the same conditions (*2*).

It seems apparent that although on the average, both free and combined riboflavin in the plasma, and the total riboflavin in the white blood cells decrease in subjects on the restricted riboflavin regimen, the variability of these values from individual to individual is so great that such analyses would be of limited usefulness in evaluating the riboflavin adequacy of either groups or individuals. It will be noted, however, that the total riboflavin content of the red blood cells not only drops considerably but consistently, on the low riboflavin intake. Therefore, it seems likely that this measurement may be useful in evaluating nutritive status with respect to riboflavin. So far, limited studies searching for factors other than intake, which might also lower the red blood cell riboflavin concentration, have been unsuccessful. It should be added that red blood cell riboflavin concentrations below 14 μg./100 ml. occur in about 2 months on a diet containing approximately 0.5 mg./day.

Conclusions

1. It seems likely that red blood cell riboflavin concentrations below 14 μg./100 ml. can be interpreted as meaning a level of intake that if continued will lead to clinical manifestations of deficiency. Experimental animals (rats) under similar conditions fail to grow normally.

2. Red blood cell riboflavin concentrations above 20 μg./100 ml. indicate an adequate riboflavin intake.

3. Data relating blood levels between 14 and 20 μg./100 ml. to intake are not sufficient to provide an interpretation.

TABLE 1

Human Riboflavin Studies

Analyses—μg./100 ml.

	Number of cases	Plasma				White cell total riboflavin	Red cell total riboflavin
		FMN+ riboflavin	FAD	Total riboflavin	Percent FMN+ riboflavin of total		
		Normal (2.55 mg. riboflavin intake/day)					
Average ± s	6	0. 90 ± 0. 48	2. 31 ± 0. 29	3. 21 ± 0. 57	28. 4 ± 8. 9	212 ± 29	22. 3 ± 2. 8
Range		0. 61 − 1. 87	1. 89 − 2. 72	2. 50 − 4. 13	22. 2 − 45. 0	175 − 245	20. 2 − 27. 6
		Deficient (0.55 mg. riboflavin intake/day)					
Average ± s	10	0. 39 ± 0. 42	2. 17 ± 0. 20	2. 56 ± 0. 70	14. 2 ± 11. 6	192 ± 33	11. 7 ± 0. 9
Range		0. 04 − 1. 43	1. 37 − 2. 91	1. 99 − 4. 34	1. 5 − 35. 0	140 − 252	10. 0 − 13. 1

4. The change in plasma and white blood cell riboflavin concentrations with change in level of intake is insufficient and too variable to be useful as a means of evaluating riboflavin status.

Thiamine

Blood thiamine.—When well-nourished albino rats (receiving 50 μg. or more thiamine daily) are placed on a thiamine-deficient diet, their red blood cell, white blood cell, and plasma thiamine content, which are normally 10–14, 50–70, and 10–14 μg./100 ml., respectively, drop to about 30 percent of these values in 2 weeks (*5*). Intermediate thiamine intakes give intermediate blood concentrations and these values can be used reliably to evaluate the thiamine status of the animal.

The red blood cell, white blood cell, and plasma thiamine concentrations of well-nourished human adults (receiving 1.0–2.0 mg. thiamine daily) are 6–9, 50–70, and 0.5–1.0 μg./100 ml., respectively (*5*). It will be noted that the plasma values in the human are strikingly lower than those for the rat. As a matter of fact, these concentrations are so low that present analytical methods are not sufficiently sensitive to provide satisfactory analyses of this phase of the blood. Another striking difference between the rat and the human, with respect to thiamine, is the fact that although in the human red blood cell thiamine decreases some with restriction of intake, this decrease amounts to perhaps no more than 15 to 20 percent, even under conditions in which clinical beriberi develops (*6*). Changes of this magnitude occur from day to day in the same individual. It seems evident that change in red blood cell thiamine concentration is not a sufficiently sensitive index of thiamine intake to be of great value in dietary evaluations.

Changes in white blood cell thiamine concentration with changes in intake in man have not been explored enough to determine whether such analyses show promise of usefulness or not.

Conclusions

Blood thiamine concentrations do not appear to be useful at the present time in the evaluation of thiamine adequacy in man.

Blood pyruvic acid.—It has been known for some years that the blood pyruvic acid concentration rises as thiamine deficiency becomes evident. However, the lack of specificity of this response has delayed its use as a means of evaluating thiamine adequacy until recently. Stotz and Bessey (*21*) showed that although blood pyruvic acid level could not be used as an index of thiamine adequacy due to the fact that the degree of activity, fasting, etc., of man and animals also influences the blood concentration of this metabolic product, they found that the ratio of blood lactic acid to pyruvic acid could serve such a purpose. Recently, Horwitt and Kreisler (*12*) have studied this procedure in great detail and have proposed what they call a "carbohydrate index (CI)"—a figure which includes the glucose concentration in the blood in addition to consideration of the lactic and pyruvic acid concentrations.

Conclusion

The lactic acid to pyruvic acid ratio or the carbohydrate index is a useful method for the evaluation of thiamine adequacy in that range of intake that approaches clinical manifestation of thiamine deficiency. For details of procedure and interpretation, the reader is referred elsewhere (*12*).

Vitamin D

It is well known, of course, that the alkaline phosphatase of the plasma rises preceding the development of rickets. It is also appreciated that concentration of this enzyme(s) varies with sex and age, increasing up through adolescence as well as in a number of disease processes, particularly those involving the skeletal system (*3*). Nevertheless, there are situations, notably in population studies where sex and age distributions can be controlled, in which phosphatase determinations would likely be of value in determining adequacy of antirachitic agents (vitamin D, sunshine, etc.).

Vitamin E

It has been established that the tocopherol concentration of plasma rises and falls with intake in both experimental animals and man. However, a number of conditions other than intake may also affect these levels (*24*). Nevertheless, it seems likely that such blood methods would find wide useful application in evaluating vitamin E status if evidence that man requires this vitamin were established.

Other Vitamins

Klein, Perlzweig, *et al.* (*13*) found no difference in the total nicotinic acid content (present mostly in the pyridine nucleotides) of the red blood cells or plasma of pellagrins and dogs with black tongue as compared with normally nourished subjects. Therefore, it would appear that blood methods cannot be expected to be useful in the evaluation of the adequacy of this vitamin.

The lack of practicable analytical methods has prevented exploration of blood methods as a means of evaluating the nutritional adequacy of folic acid, pantothenic acid, vitamin B_6, and vitamin B_{12}.

General Conclusions

There are available practicable chemical methods adequate for analysis for a number of the vitamins in 1 or more of the 3 phases of blood (red blood cells, white blood cells, and plasma).

There is an established correlation between blood content and intake for a number of the vitamins over the range of intake important in considerations of adequacy. In these instances, when properly used and interpreted, blood analyses are useful in evaluating the nutritive status with respect to a specific nutrient.

Due to the intrinsic nature of such analytical methods and the biochemistry of the vitamins, there are limitations in the application of such methods. Unless these limitations are ever kept in mind, disappointing results from their use will occur.

In general, the methods are more reliable when used in group studies. However, with proper application, presumptive information about individual status is readily obtainable.

There is serious need for detailed measurements of blood concentrations of groups of individuals maintained on varying and well controlled levels of intake of each of a number of the vitamins in order to establish more completely and reliably the relationship between these two variables. At the present time, this relationship can be only roughly expressed because it is derived from the piecing together of small bits of data from a number of sources and heterogeneous experimental conditions.

There is also need for further study to establish clearly those factors other than dietary intake that affect blood vitamin concentrations. This is especially necessary before the use of such methods can be expected to give reliable results in attempts to evaluate adequacy in pathological states.

Finally, there is that very difficult problem of the need for more complete and adequate methods and measurements of the health status that results from a given level of vitamin intake, for until such information is available, the interpretation of blood analyses as well as of all other types of analyses that measure dietary intake will have many areas of uncertainty and controversy.

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Evaluation of Vitamin Adequacy: Urinary Excretion Tests

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Measurement of the urinary excretion of many of the vitamins, or their metabolites, has been widely used in evaluation of nutritional status of individuals and population groups. Tests have been carried out with urine specimens collected for 24 hours, for 1 hour during fasting, for varying periods of time after oral or parenteral administration of a test dose of the vitamin, and in random specimens. In general, the concentration of essential nutrients in urine reflects recent dietary intake but numerous conditions not primarily related to nutrient supply influence findings. Tests conducted for 24-hour periods or longer are more informative than those involving shorter intervals of time. Examination of random specimens, relating vitamin excretion to creatinine content, has proved useful in surveys.

Excretion of certain vitamins following administration of a test dose reflects, to an extent, the degree of saturation or depletion of body tissues. Factors which may influence findings include variations in renal function, volume of urine, completeness of evacuation of the bladder, and, in oral tests, rate of absorption. Marked variations of excretion by subjects on the same diet has been observed. Recent access to the nutrient in question may affect results. In starvation, or in pathologic conditions associated with breakdown of tissues, unexpectedly large amounts of certain vitamins or their metabolites may be excreted. The general character of the diet and interrelationships among nutrients influence utilization, and hence affect output in the urine.

The vitamins for which urinary excretion tests have been studied extensively include thiamine, riboflavin, niacin, and ascorbic acid. A few investigations of urinary excretion of vitamin B_6 metabolites, pantothenic acid, folic acid, and vitamin B_{12} have been reported. Evaluation of tests for each of these factors will be discussed.

Thiamine

Thiamine excretion in 24-hour specimens of urine is linearly related to the intake except at low levels, although intake-excretion curves differ with experimental conditions (*21*, *35*). Excretion is also characteristic of the individual, varying considerably among persons receiving identical diets. When the dietary supply of thiamine is decreased to less than 1 mg. daily, excretion falls rapidly at first, and then more slowly until equilibrium in urinary excretion is attained (*7*, *22*). Minimal levels of excretion in the neighborhood of 1 μg. to 10 μg. daily may be reached at levels of intake below 0.6 mg. in adults (*35*).

In studies of experimental thiamine depletion, early clinical signs of deficiency have been reported when the urinary excretion in 24 hours was less than 40 μg. daily, the dietary intake being 0.35 mg./1000 cal. (*10*), and when the urinary excretion was 5 μg. to 20 μg., the dietary intake being 0.17–0.21 mg./1000 cal. (*12*). In beriberi, excretion of 0 μg. to 14 μg. has been noted (*24*). The level of excretion at which deficiency appears would seem to be dependent on dietary intake, time, and individual variation.

Determination of excretion in 24 hours appears to be useful in reflecting the dietary supply, particularly of groups of individuals. If mean excretion is low, potential or actual thiamine deficiency may be suspected.

Lowry (*32*) has proposed the use of random specimens in field studies, as a screening test, measuring thiamine excretion in relation to creatinine output. Louhi and associates (*31*) found that the ratio of thiamine to creatinine was fairly constant in voided specimens throughout the day and could be used for rough estimation of thiamine nutrition. Since a small adult will excrete approximately 1 gram of creatinine daily, Lowry suggested that an excretion of 100 to 150 μg. of thiamine per gram of creatinine would not appear to be compatible with deficiency. Application of the above procedure in surveys in Newfoundland, before and after flour enrichment (*1*), and in Bataan, before and after rice enrichment (*6*), indicated that thiamine excretion per gram of creatinine reflected a change in the thiamine supply of these population groups.

Pollack and coworkers (*42*) and Mickelsen and associates (*35*) have suggested that determination of the urinary excretion of pyrimidine degradation products of thiamine may be more useful in evaluation of thiamine nutrition than estimation of excretion of thiamine *per se*. Since few studies of pyramin excretion have been carried out, the usefulness of this procedure cannot be appraised.

Thiamine excretion in the urine during fasting has been found to correlate with average dietary intake and with the 24-hour urine excretion (*16*). It has been suggested that excretion of 0 μg. to 4 μg. of thiamine in a 1-hour specimen is indicative of deficiency. The criteria for diagnosis of deficiency have varied among investigators. In the Philippines, mean thiamine excretion in 1 hour was found to be 6.1 μg. in apparently normal persons, 2.7 μg. in subjects with obvious or questionable beriberi (*48*). Overlapping of values among the three groups indicates that determination of thiamine excretion during fasting has limited usefulness as a diagnostic test. The test would appear to give some information as to previous dietary intake.

The excretion of thiamine following administration of oral or parenteral doses of the vitamin decreases as dietary thiamine is lowered in persons on controlled intakes. There is disagreement as to the level of excretion which is indicative of deficiency. Interpretation of findings is even more uncertain in persons on varied diets. Mean thiamine excretion in 4 hours following an oral dose of 5 mg. has been found to be lower in patients with signs of vitamin-B complex deficiency than in normal subjects but there is no clear line of division between the two groups (*16*). Lossy and associates (*30*) reported a mean 4-hour excretion of 214±18 μg. in normal subjects, 53±12 μg. in patients with peripheral neuritis or signs of beriberi heart disease, and 49 ±14 μg. in subjects without signs of thiamine deficiency but receiving diets containing 0.5 to 0.8 mg. of thiamine. Thus, this "load" test will reflect dietary intake but is not diagnostic of clinical deficiency. The accuracy with which it reflects body stores is uncertain.

Oldham and associates (*38*) found that urinary excretion of thiamine in 4 hours after an oral dose of 20 μg. per kg. of body weight was a reflection of dietary intake and a potential measure of tissue stores.

A procedure which has been studied widely is measurement of urinary thiamine excretion for 4 hours after parenteral administration of 1 mg. of thiamine (*16*). An excretion of less than 50 μg. is generally considered indicative of thiamine deficiency; an excretion of 100–200 μg. of adequate thiamine intake. Most of the studies with this test have been made in subjects on constant diets. Wider application of this procedure in field work would seem desirable.

A variation of the above test, using 350 μg. of thiamine per square meter of body surface, has given results of similar nature. However, low excretion has been observed in debilitated persons regardless of the etiology of their condition and an increase in excretion during convalescence (*16*).

Good correlation between all of the urinary excretion tests mentioned above has been reported.

From the information at hand, it seems probable that a load test will prove to be a more satisfactory index of tissue thiamine stores than either fasting or 24-hour urinary excretion. Lowry (*32*) has proposed that tissue deficit may be measured by repeated administration of large divided doses of thiamine, given parenterally since large oral doses may be poorly absorbed. Studies with radioactive thiamine should elucidate further the metabolism and excretion of this vitamin.

Riboflavin

The relationship between dietary intake and urinary excretion of riboflavin has been studied extensively. When the diet furnishes less than 1 mg. of riboflavin daily, urinary excretion represents 9 to 14 percent of the intake (*16*); when the dietary supply is above 1 mg. per day, excretion in the urine increases to 30 percent or more of the intake (*23*, *28*). While Horwitt and associates (*23*) found this relationship to be definite when mean results were considered, standard deviations were large. Accordingly, individual values must be interpreted with caution. It has been suggested that the sharp rise in riboflavin excretion at intakes above 1 mg. may indicate approximate tissue saturation, although other explanations are possible (*32*). Further investigation by tissue analysis or with isotopically labelled riboflavin will be necessary for elucidation of findings.

Experimental riboflavin deficiency has been induced in subjects receiving diets which furnished less than 0.6 mg. of riboflavin daily. In one study, mean urinary excretion of riboflavin was 77 μg. in 24 hours, in another less than 50 μg. (*16*). Excretions of less than 50 μg. of riboflavin daily have been reported in association with glossitis and ocular lesions of riboflavin deficiency (*16*). Although an excretion of less than 100 μg. of riboflavin has been observed during deficiency, excretions of the same order may be found in the absence of clinical lesions. There is a wide variation in excretion among individuals receiving similar diets.

While no specific level of riboflavin excretion can be suggested as diagnostic of clinical deficiency, an excretion of more than 200 μg. in 24 hours is seldom associated with significant tissue depletion. Exceptions to this statement may be cited. The output of riboflavin is increased in acute starvation (but not in chronic) (*28*), in diabetes (*30*), in conditions associated with negative nitrogen balance (*41*), and after administration of certain antibiotics (*14*). An increase in excretion was observed in young men on a diet deficient in thiamine but a decrease occurred when the diet was deficient in both thiamine and riboflavin (*28*). Physical activity also influences excretion (*28*).

Several methods of evaluating riboflavin nutrition under conditions of field surveys have been suggested. Determination of excretion in a 1-hour specimen of urine collected during fasting has been proposed but not widely applied (*37*). Interpretation of findings are subject to the limitations noted above.

Lowry (*32*) has measured riboflavin excretion in random non-timed specimens in relation to creatinine excretion. He suggested that an excretion of more than 150 μg. of riboflavin per gram of creatinine was indicative of saturation. This test was used in surveys in Newfoundland in 1944 and 1948 (*1*). Findings reflected dietary changes although no correlation was demonstrated between clinical signs suggesting riboflavin deficiency and urinary excretion of this vitamin.

A number of load tests have been devised for appraising riboflavin nutrition. Measurement of excretion for 4 hours following oral administration of a 5 mg. test dose has been proposed by several investigators (*37*). Lossy and associates (*30*) found this test to be satisfactory in differentiating between subjects on good diets and those on weighed diets low in B vitamins but not between control subjects and patients with signs of vitamin-B complex deficiency. In several studies riboflavin has been administered orally or parenterally in amounts of 2 mg. or less and urinary excretion determined during 4- or 24-hour periods. In subjects maintained on constant diets, excretion following the load test reflected dietary intake (*16*). Horwitt and associates (*23*) studied the response to a test dose of 1 mg. given subcutaneously, at eight levels of riboflavin intake. The excretion in 4 hours was below 100 μg. when the intake was 1.1 mg. or less and the standard deviation was small; at higher levels of intake, wide variations in excretion were observed.

It seems logical to suggest the use of a small test dose in evaluating riboflavin nutrition in view of the marked rise in the urinary excretion of this vitamin at dietary levels above 1 mg. The use of a 1 mg. parenteral dose with estimation of excretion in the subsequent 4 hours would appear to merit further study.

Niacin

Chemical tests for evaluation of niacin nutrition have been unsatisfactory until recently for a number of reasons. Niacin is excreted in the urine largely as N^1–Methylnicotinamide (N^1–Me.) and the 6-pyridone of N^1–Me. (pyridone). Methods for measurement of pyridone have been developed only in the past few years and are still time-consuming. The fact that tryptophane serves as a precursor of niacin in the body necessitates estimation of the tryptophane, as well as the niacin, content of diets in studying relationships between excretion of niacin metabolites and the intake of this vitamin. More information is needed as to the ratio of conversion of tryptophane niacin.

Niacin nutrition has been studied by estimation of N^1–Me. excretion in 24 hours, in 1 hour during fasting, and for varying periods of time after administration of an oral test dose of niacinamide. More recently, excretion of pyridone has been measured as well. Excretion of N^1–Me. in 24 hours has been found to decrease to low levels in patients with pellagra and in subjects maintained on diets low in niacin and protein (*16*). A low or zero excretion of N^1–Me. during fasting was reported to be indicative of low niacin stores. However, some normal persons fail to excrete appreciable amounts of N^1–Me. in the postabsorptive period. A large excretion of N^1–Me. has been noted in prolonged fasting and in association with diseases characterized by severe muscle wasting (*16*).

A number of investigators found that urinary excretion of N^1–Me. after an oral test dose of 50 to 500 mg. of niacinamide was lower in patients with signs of vitamin-B complex deficiency than in normal subjects (*16*, *30*). However, patients hospitalized for miscellaneous conditions also showed a low excretion after the test dose.

Recent studies indicate that excretion of pyridone decreases more rapidly than that of N^1–Me. in persons receiving diets low in niacin and tryptophane (*19*, *46*). Patients with pellagra or experimental niacin deficiency excrete only traces of pyridone while N^1–Me. excretion remains in the neighborhood of 0.5 to 0.8 mg. daily (*17*, *19*). Goldsmith and associates (*18*) supplemented corn diets containing 200 mg. of tryptophane with increments of niacin so that the diets furnished 4.6 to 21.2 mg. of niacin daily. Excretion of niacin metabolites (N^1–Me. and pyridone) increased sharply when the daily intake was between 8 and 10 mg. (*17*, *18*). Below this level, about 0.2 mg. of niacin metabolites were found in the urine for each mg. of niacin ingested; above this level, the excretion rate was 0.6 mg. of metabolites for each mg. of niacin in the diet. In the light of these findings, it was suggested that with the corn diet, which furnished 200 mg. of tryptophane, body niacin stores are adequate when the niacin intake is 8 to 10 mg. daily.

Following administration of a test dose of 100 mg. of niacinamide, Holman and deLange (*20*) found that the major increase in N^1–Me. excretion occurred within the first 3 hours, values falling to control levels after 9 hours. Maximum pyridone excretion was observed in the second 3 hours and decreased slowly throughout a 24-hour period. It would seem desirable, therefore, to determine urinary excretion of niacin metabolites for at least 12 and preferably 24 hours after a test dose rather than for shorter periods of time. Rosenthal and associates (*46*) found that N^1–Me. and pyridone excretion in 24 hours was lower in patients with pellagra than in normal subjects, both on standard diets and following oral administration of 10 or 25 mg. doses of niacinamide. Measurement of excretion after a test dose of 50 mg. of niacinamide was not satisfactory in differentiating between normal and deficient subjects.

Another test which is being explored currently is measurement of niacin metabolites in the 24-hour urine when 10 mg. of niacinamide is given daily to subjects on a standard diet which furnishes 10 mg. of niacin and 1,000 mg. of tryptophane (*17*). The time required to obtain maximum excretion with this regimen may reflect nutritional status relative to niacin.

In further development of tests for evaluation of niacin nutrition of individuals, two procedures appear to warrant additional investigation: (a) determination of both N^1–Me. and pyridone excretion in the urine in 24 hours on a standard diet, and (b) measurement of the excretion of these metabolites in 24 hours after administration of a small (10 mg.) oral test dose. In surveys, it is suggested that N^1–Me. and pyridone excretion be measured in relation to creatinine output in random specimens of urine.

Ascorbic Acid

Tests for evaluation of ascorbic acid nutrition include measurement of excretion in 1 hour during fasting, in 24 hours, and for varying time intervals after administration of oral or parenteral doses of 100 to 1,000 mg. of ascorbic acid (*16*, *37*). Studies have been carried out in normal subjects, both on constant and varied diets, and in patients with scurvy.

The method most widely used for determining the vitamin C content of urine, direct titration with 2,6–dichlorophenolindolphenol, is relatively nonspecific. In subjects deprived of ascorbic acid for several months, values for urinary excretion obtained with this procedure ranged from 13 to 48 mg. daily (*34*). Reducing substances other than vitamin C must largely account for such findings. The method is useful when the quantities excreted in the urine are above 50 mg., for example, in saturation tests. The modifications of this technique proposed by Evelyn and associates (*11*) eliminates some of the error due to the nonspecific reducing substances. The dinitrophenylhydrazine method of Roe and Kuether (*45*) has relatively high specificity, as does a method developed by Mapson (*33*), which was used in the British Medical Research Council's recent study of vitamin C requirement (*34*). In the future, procedures of high specificity should be applied.

The renal mechanism for excretion of ascorbic acid has been the subject of much investigation. Friedman and associates (*13*) found that when the concentration of ascorbic acid in plasma was less than 1.4 mg. percent, the plasma clearance was approximately 1.5 ml. per minute. When the ascorbic acid levels in plasma exceeded 1.4 mg. percent, clearance rose rapidly, reaching about 40 ml. per minute at 2 mg. percent. In view of this favorable renal mechanism, and the high percentage of large doses of ascorbic acid which may be recovered in the urine, it is possible to estimate the degree of unsaturation of the tissues if flooding of the plasma is avoided. With these findings in mind, Lowry (*32*) has proposed measurement of tissue deficit of ascorbic acid by administering large amounts, 500–2,000 mg. daily, in divided doses to avoid raising the plasma level above 1.4 mg. percent, and determining the quantity of the vitamin excreted in the urine. Tissue retention should equal tissue deficit when suitable correction is made for destruction of the vitamin. This test is more informative than the usual load test but is applicable only under carefully controlled conditions.

In many clinical studies and in surveys, the usual type of load test has been performed (*16*, *37*). Unfortunately, there has been no standardization as to size of test dose, route of administration, or period of urine collection. Few studies have been carried out in subjects on controlled diets. In many instances, criteria for the grouping of subjects as to adequacy of vitamin C nutrition were arbitrary and poorly justified, thus influencing interpretation of results. In spite of these difficulties, findings in the various load tests have reflected, in general, dietary intake and tissue stores of ascorbic acid. If continued use of such tests is contemplated, it would be desirable to outline a standard procedure which should then be studied under conditions of known ascorbic acid intake. An attempt to correlate findings with ascorbic acid concentration in the white cell-platelet layer of blood would be valuable.

Measurement of urinary excretion of ascorbic acid in 24 hours may be of assistance in estimating dietary intake of vitamin C. However, unless sufficiently large groups are studied, individual variations may distort findings. When the ascorbic acid intake is 100 mg. or more daily, approximately 60 to 80 percent is recovered in the urine; when the intake is 40 to 100 mg. 20 to 60 percent may be recovered (*32*). When the diet furnishes only small amounts of ascorbic acid, excretion may not reflect differences in intake (*34*). In surveys, determination of excretion in the postabsorptive state, or in random specimens, basing ascorbic acid output on creatinine excretion, should give information similar to that obtained from estimation of excretion in 24 hours.

It should be emphasized that ascorbic acid nutrition is affected by many types of acute and chronic illness such as infections, burns, other traumatic and stress situations, and by certain drugs. Under these circumstances, findings in chemical tests will not be a reliable measure of ascorbic acid intake.

Vitamin B_6

The predominant vitamin B_6 metabolite excreted in urine, either normally or following the administration of pyridoxine, pyridoxal, or pyridoxamine, is 4-pyridoxic acid (*25*, *29*, *43*). Subjects on self-selected diets excrete an average of approximately 35 mg. of 4-pyridoxic acid in urine in 24 hours (*25*, *36*, *43*, *50*).

Relatively little is known concerning the amount of this substance excreted at various levels of vitamin B_6 intake. Data reported by various investigators may be subject to errors introduced by lack of specificity of the technique employed for the determination of 4-pyridoxic acid in urine (*50*). Such errors would be greatest at low levels of intake and excretion, and may account for the reported excretion of amounts in excess of the dietary intake (*29*). Data obtained regarding urinary excretion of 4-pyridoxic acid following test doses of vitamin B_6 would not necessarily be invalidated, as interference with the determination by other substances would be minimized by dilution (*50*).

Greater quantities of 4-pyridoxic acid are found in the urine after the administration of pyridoxal than after pyridoxine or pyridoxamine (*43*, *50*), the greater portion of excretion occurring within 5 hours. Rabinowitz (*43*) found that 70 percent of a 100 mg. test of pyridoxal hydrochloride was excreted in the urine of normal subjects within 24 hours of its administration. Lossy and associates (*30*) found that normal subjects excreted 9.5±0.4 mg. of 4-pyridoxic acid in the urine in a 4-hour period following the oral administration of 25 mg. of pyridoxal hydrochloride. Subjects on diets low in B-complex vitamins, and patients with signs of thiamine or B-complex deficiency showed no significant difference in urinary excretion from the normal group.

In the light of present information, determination of vitamin B_6 metabolites in urine shows little promise as a test of adequacy of vitamin B_6 nutrition.

Pantothenic Acid

Although pantothenic acid deficiency has not been observed in man, many investigations of the urinary excretion of this vitamin, under varied dietary conditions, and following oral and parenteral load tests, have been reported (*8*, *9*, *38*, *39*, *40*, *47*, *49*, *52*). No statement can be made regarding the applicability of these procedures in the evaluation of pantothenic acid nutrition.

Folic Acid

The evaluation of folic acid nutrition in human subjects is complicated by incomplete knowledge of the distribution of this vitamin in foods, and by lack of information concerning the relative activities for man of its naturally occurring conjugates.

Folic acid depletion has not been produced experimentally in man. There is little information regarding urinary excretion of the vitamin under controlled conditions or following the administration of test doses. The majority of studies reported indicate that normal subjects on "normal" diets excrete between less than 1 μg. and 10 μg. of folic acid in the urine daily (*8*, *15*, *44*, *54*, *55*, *56*).

In addition to folic acid, citrovorum factor, which is believed to be a metabolically active form of the vitamin, is found in normal urine (*15*, *44*). Following oral or parenteral administration of folic acid, a small portion of the dose appears in the urine as citrovorum factor (*5*, *15*, *27*, *51*, *54*).

Data reported by Bethell (*3*) and Swendseid (*56*) suggest that patients with pernicious anemia excrete less folic acid in the urine in 24 hours than do normal subjects maintained on the same diet. Girdwood (*15*), however, observed no significant difference in the 24-hour urinary folic acid excretion of pernicious anemia patients and nonanemic control subjects.

The oral or parenteral administration of folic acid to normal subjects results in the urinary excretion of amounts of the vitamin which vary roughly with the size of the dose administered, the greatest excretion occurring within 6 hours (*15*, *26*, *30*, *53*, *56*). It has been reported that patients hospitalized for miscellaneous illnesses excrete less of a test dose than do normal subjects (*30*, *55*). Patients with pernicious anemia and the sprue syndrome excrete significantly smaller amounts of folic acid and citrovorum factor following a test dose of folic acid than do normal subjects (*3*, *15*, *54*, *56*).

Broquist (*5*) has reported that administration of ascorbic acid with folic acid as a test dose increases the amount of citrovorum factor excreted in the urine. However, a recent study suggests that this is not a specific effect of ascorbic acid (*4*).

Until more information is available, estimation of folic acid excretion in the urine would appear to be unsatisfactory for evaluation of folic acid nutrition.

Vitamin B_{12}

The daily dietary requirement of vitamin B_{12} is not known, and relatively little information regarding its quantitative distribution in foodstuffs is available. Techniques for determination of the vitamin vary greatly in sensitivity and specificity, and as a result, there is no general agreement on the amount of the vitamin excreted in the urine by normal subjects or by patients with clinical syndromes known to respond to therapy with vitamin B_{12} (*57*).

Measurements of urinary excretion of vitamin B_{12} following oral or parenteral administration of a wide range of test doses indicate that little or none of the vitamin is found in the urine following oral administration (*57*), and that the amount excreted following intromuscular injection increases roughly in proportion to the size of the dose administered (*2*, *57*, *58*). Whether any of these procedures will be found useful in appraisal of nutritional status remains to be determined.

Summary

Urinary excretion tests are of value in the appraisal of nutritional status of human subjects in regard to thiamine, riboflavin, niacin, and ascorbic acid, under certain conditions.

Estimation of excretion of these vitamins in a 24-hour period reflects dietary intake. This procedure is more valuable when applied to population groups than to individual subjects. Determination of vitamin excretion, in relation to creatinine output, in random specimens of urine appears to give information comparable to that obtained from excretion in 24 hours and is especially applicable in surveys. Load tests which appear to offer promise in evaluating tissue stores of these vitamins are as follows: For thiamine or riboflavin, measurement of urinary excretion of the respective vitamin for a 4-hour period after parenteral administration of a 1 mg. dose; for niacin, measurement of urinary excretion of N^1–Me. and pyridone for 24 hours on a standard diet with or without administration of a test dose of 10 mg. of niacinamide. Perhaps a 12-hour excretion test for this vitamin will prove feasible. There is need for standardization of a load test for ascorbic acid, although most of the tests now in use are reasonably informative. When tissue stores of the above vitamins are severely depleted, tissue deficit may be estimated by administration of appropriate daily doses and measurement of excretion in 24-hour periods.

Excretion tests have not been proved valuable, as yet, for appraisal of vitamin B_6, pantothenic acid, folic acid, or vitamin B_{12} nutrition.

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Some Considerations in Making Therapeutic Trials

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Medical therapeutics has developed from its infancy of empirical use and dosage to an exact pharmacological science based upon biochemical, physiological, and statistical reasoning and dependent on clinical acumen, laboratory data, and controlled observation. The abuse of the empirical use of antibiotics is only too painfully evident in the resistant bacteria that we as physicians have produced. We have used penicillin like water. The result has yielded resistant organisms and hypersensitivity reactions. It is no more disturbingly true than with the haemolytic staphylococci aureus. This organism is now 100 percent resistant to penicillin and has even developed resistance to newer antibiotics. In vitamin therapy we have been still more gullible. Vitamin pills have been dispensed by the bucket. Fortunately, we have not a serious problem in resistance to or toxicity of these drugs. However, toxicity has occurred in the instance of vitamins D and A and much of the administration of all vitamins is on an even more confused therapeutic basis than is the use of penicillin for every common cold.

The result of empirical abuse of therapeutic agents is bringing about a somewhat more realistic approach to the clinical use of certain drugs. Is this true in the field of nutrition? If not, we need but pause for reappraisal of our facts in order to make it so. We have behind us 15 years of confusion and exploitation of nutrition with its associated empirical abuse of vitamin supplementation. Are we not now sufficiently mature that we can avoid the fiascos of the too-recent past in which each new factor has been endowed with such glamour that it served to "cure" those same diseases in which the faith-healer is proficient or which have notoriously undulating courses?

It is evident that thiamine, niacin, and vitamin C have specific therapeutic effects in beriberi, pellagra, and scurvy. No one can argue the value of therapeutic trial in the frank vitamin deficiency status. The response is prompt and curative. It is not our purpose to dwell upon the appraisal of treatment in these conditions. Simply designed trials may prove lifesaving under conditions of acute deprivation, and the prompt response obtained is too evident to mislead seriously. It is in the field of so-called "subclinical vitamin deficiencies" that we feel there is a need for more critical thought and therapeutic testing.

There is a basic design for therapeutic trials. Several workers, among whom may be mentioned Daniels (*4*) and Bean (*3*), have set down certain principles of the testing of new drugs and of rations. It would be well for us to adapt these methods to the therapeutic testing of vitamins. Some of the important considerations may be discussed. The clinical trial from the beginning must be properly designed to give answers to specific questions, organized so that required information is available for all cases, and planned to allow statistical analysis from a collection of unequivocal, unbiased data. The sample must be large enough, and should be representative of the population to which it belongs. Intentional or unintentional bias must either be eliminated or recognized. The criteria of selection of cases or subjects and the types of treatment must be clearly defined. The routine and uniformity of examination must be established prior to the therapeutic trial. "Control groups" serve as real controls only if they are arranged through proper use of the placebo. In this instance, all observers and workers who deal with the subjects must be ignorant of the identity of the placebo as well as identity of the groups. Uniform worker-patient contact must be established.

The criteria of change must be clearly defined. Are these subjective on the part of the observer or trial group? Are they observational or chemically measured changes? Would any of us suggest initiating and evaluating treatment of anemia on the basis of the pallor of the conjunctiva or fingernail beds? Failure to employ chemical and quantitative physiological measurements in therapeutic testing of vitamins is, in many instances, equally outmoded. Measurements of ascorbic acid levels, of serum vitamin A, of urinary excretion of water-soluble factors, etc., are so commonplace that it is ordinarily inexcusable not to use such laboratory measures except where one is working under the exigency of field conditions, prison camps, and the like. Photographic evidence cannot substitute for these, because it may be misleading. A change of angle or intensity of lighting has been known to convert a normal tongue into a better magenta one than is ever observed to result from ariboflavinosis.

Before setting about upon a therapeutic trial, it is essential to define the general conditions of the patients or groups. (See table 1.) Are intercurrent infection, gastro-intestinal disorders, and the like going to influence the results? What of the geographic, socioeconomic and other environmental factors? Are they likely to prejudice the trial?

What of the dietary intake? It is basic that we know the nutritional intake of our test groups. One cannot expect to detect clinical or bio-

TABLE 1

AVERAGE HEMOGLOBIN CHANGES (gm.) IN INFESTED CHILDREN RECEIVING ANTHELMINTIC WITHOUT AND WITH SUPPLEMENTARY IRON

Original hemoglobin (gm.)	Anthelmintic alone			Iron and anthelmintic		
	1–2 months	12 months	18–24 months	2 months	10–12 months	24–36 months
0.0–5.9	0.3	3.2	6.5	3.4	4.3	6.3
6.0–6.9	1.1	--------	5.0	2.8	3.7	4.4
7.0–7.9	1.3	4.2	4.4	1.4	2.7	3.5
8.0–8.9	1.4	3.1	4.1	2.2	2.9	3.8
9.0–9.9	1.0	2.5	2.8	2.0	2.8	2.8
10.0–10.9	1.0	2.2	2.1	1.2	1.8	2.4

After Payne and Payne, *Am. J. Hygiene*, *32*, 125 (1940).

chemical changes of any significance when the nutrient intake is abundant prior to vitamin supplementation, even though so-called evidences of "subclinical deficiency" are present.

Finally, the results of the trial must be searchingly analyzed, preferably by disinterested statisticians who examine the internal comparability of the observations. The analysis should be limited to data that concern measurable facts. There cannot be disagreement about facts so obtained.

The interpretation of these facts may vary from one observer to another and, hence, lead to differences of opinion. The resultant differences yield discussion. Discussion may lead to progress and solid conclusions, or to temporary compromise judgments followed by recognition of the need so frequently expressed in the last paragraph of articles in *Nutrition Reviews*—the need for further study.

On the other hand, where attempts are made to support theories by means of our therapeutic trial, things are different. As Maurice Arthus (*1*) so delightfully puts it in the preface to his "De l'Anaphylaxie a l'Immunite." . . . . "A theory is a dogma, based on some arguments of faith. . . . Does it correspond to reality, to truth? . . . . Perhaps, but we are never sure about it . . . . In accepting it, we perform an act of faith and in rejecting it we perform an act of doubt . . . . One says and repeats that the theory is only provisional . . . . But in reality, those who repudiated a theory they once proposed or enthusiastically accepted are very rare . . . .

"The great theories go beyond the boundaries of the laboratory. They are usually announced in scientific periodicals, the daily newspapers, etc., in glowing language. The theoreticians find themselves in the limelight of publicity. How could they change their opinion without losing face?

"And then we see a performance that is pathetically sad . . . . Blinded by his passion . . . . his immoderate love for his theory, he has recourse to all means, honourable or not . . . . in order to defend it . . . . He has ceased to be a scientist and has become a partisan." Arthus' final advice, then, is:

"Seek facts and classify them—and you will be the workmen of Science. Conceive or accept theories—and you will be their politicians."

The facts in assessing the value of a specific nutrient in so-called "subclinical states" are difficult to establish due to the reliance upon subjective ratings and the too frequent omission of objective quantitative measurements. Most signs of avitaminosis are not specific and may have multiple nutritional etiologies as well as non-nutritional ones. Thus, the signs most often used as evidence of clinical ariboflavinosis are recognized as resulting from a very large number of conditions, including climatological and dental. In some instances, nutrition may be but one component which may bear on the occurrence of a physical sign. For example, oral hygiene seems more important than ascorbic acid status in determining the existence of common gingivitis or its healing.

Lawyers have long recognized that two witnesses "see" happenings differently, even under oath. Visual observation is disturbingly difficult to separate from one's background, educational experience, or conditioning. That physicians, as observers, suffer from this inability for complete objectivity has been well documented by Bean (*2*). Bean found significant variation in the observations by a group of physicians trained in clinical nutrition and who were using a set of "objective standards" during examinations of soldiers for signs possibly indicative of nutritional deficiency.

These same human failings have been uncovered in our extensive study of nutrition in pregnancy (*5*). Our analysis of clinical signs indicate that as one might anticipate, the incidence of these was influenced by age, parity, season of the year, stage of gestation, and examiner. The latter exerted the greatest influence in several instances. A humorous example of this concerns the examiners' ratings of the patients as obese, normal, or undernourished. Thin examiners found more obesity and less undernutrition than did those physicians who were themselves overweight or who had "pleasingly plump" wives. (See table 2.) It is apparent that, in spite of efforts to develop objectivity, one cannot regiment judgment.

Unfortunately, the use of a single observer is no guarantee of constancy of subjective rating. Over a period of time, the experiences of the observer may alter his criteria. The tendency to rate findings on a relative basis within a group is recognized by all of us who have had the experience of working for some time with a large sample at one end

TABLE 2

PHYSICAL FINDINGS BY EXAMINER EXPRESSED AS PERCENT OF TOTAL EXAMINATIONS BY THE EXAMINER

Examiner	General nutriture	
	Under	Obese
R. C	26. 9	9. 5
K. S	24. 3	12. 6
E. W	11. 4	19. 8
C. W	8. 4	16. 9
W. Mc	14. 7	27. 2
R. P	3. 1	13. 8

of the nutritional scale and then shifting to another sample at the opposite extreme.

Another feature of the therapeutic trial that is open to speculation is the duration of therapy. Where, in the field of medicine—aside from vitamins—would one give specific medication for months and years with no clinically detectable improvement and have the courage to state that the drug may still be beneficial? It seems incongruous that in frank vitamin deficiencies, which in areas of the world where these are prevalent must occur in chronically malnourished subjects, the therapeutic response to specific vitamins is prompt and dramatic. Yet, with the so-called "subclinical" signs of avitaminosis, the lack of therapeutic response is often attributed to an insufficient length of treatment. Scars and irreversible stigma of malnutrition do occur, such as skeletal deformities from rickets, serious goiter from iodine lack, etc. But these findings, sufficiently documented to withstand inquiring scientific examination, have resulted from rather severe states of recurrent deficiency of obvious clinical degree.

Despite this, we would not advise abandoning efforts to learn the effect of therapy upon slowly altering lesions. Instead, we plead for recognition of the pitfalls of investigation during the design and execution of the study. The natural history of the disease in the particular setting and sample must be recognized. To illustrate: Keratosis pillaris and similar dry, mildly hyperkeratotic lesions of the skin soften and become less noticeable during warm months and more apparent in the cold season. A therapeutic trial of "X nutrient" instituted in November and discontinued in August might lead to some striking data supporting the "effectiveness" of a totally useless agent.

In studying the protection against relapse offered by an agent, one might also be misled by the nature of the disease. Take the example of pernicious anemia: withdrawal of maintenance liver extract therapy may be followed in some patients by excellent health and satisfactory hematological levels for periods up to 5 years. A few

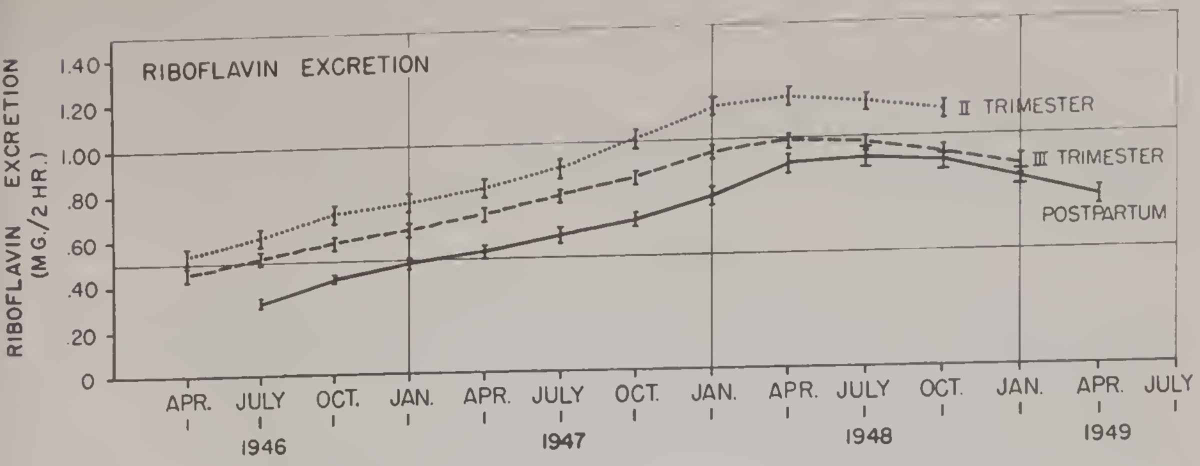

FIGURE 1.—Five years of study on obstetrical patients shows gradual increase in level of riboflavin status on urinary excretion.

such patients treated with an inactive substance could trap the unsuspecting investigator.

Finally, in long-term therapeutic trials, it must ever be borne in mind that we are studying the human. Man's station in life, his educational experience, income, dietary habits, and so on change—sometimes insidiously. These attributes in groups also may vary. To illustrate, during the nearly 5 years of continuous study of the population of obstetrical patients attending the Vanderbilt University outpatient clinic a gradual increase in level of riboflavin status seemed indicated by data on urinary excretion. (See fig. 1.) If such trends coincided with alterations important to the therapeutic test at hand, they could be quite misleading. Since one cannot control the habits of human subjects, we must measure those characteristics which may be especially important, in order to take them into account in interpreting experimental data. If this cannot be done, the limitations imposed by lack of such information must be recognized.

Lastly, one comes to the considerations which we place first in our thoughts concerning any research on the human, including the therapeutic trial. What is the ethical justification for a particular investigation? What benefits may accrue to the subjects under study or to other patients as a result of the study? Is there a hazard involved due to the agent to be administered or to the restrictions or procedures required for completion of an experiment designed to evaluate the test substance? Do the conditions which make possible the critical control of the human subjects arise under circumstances which offend our sense of moral and economic responsibility? Is there a member of the investigative team who is qualified to appraise the unexpected situations which may arise? These and related considerations must be answered by each group of responsible investigators. There can be no generally applicable formula for determining the answer.

Nutritional testing dealing with several levels of disease is possible. The tremendous progress in understanding experimental design and in defining methodology during the past 15 to 20 years should be used in the construction of such studies. We have raised for your attention some of the considerations involved. Properly designed therapeutic trials will serve as widening paths toward truth; improperly designed trials will lead us deeper into the overgrown jungle of confusion.

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Vitamin Adequacy and Specific Metabolic Functions

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Today I would like to examine certain aspects of the problem of how variations in vitamin intake affect specific metabolic functions in living organisms. Time prohibits anything approaching a complete review of this topic; I have chosen instead to discuss it in the light of experimental findings with micro-organisms, pointing out, where possible, the obvious corollaries that may exist in animal nutrition. Most of these experimental findings will deal with a single nutrient, vitamin B_6.

Recent years have seen rapid advances in our knowledge of how various vitamins function in metabolism. In every case so far examined, a derivative of the vitamin functions in combination with one or more proteins to form a conjugated protein is required for promotion or catalysis of some bodily function. Most commonly, the vitamin derivatives in question are the *coenzymes*, the proteins are the corresponding *apoenzymes*, and these combine to yield the fully functional *holoenzymes*, required for catalysis of a great variety of chemical reactions within the cell.

Suboptimal intake of an essential vitamin obviously should result in decreased synthesis, and hence to a decreased tissue concentration of those coenzymes derived from the vitamin. Thus the vitamin B_6 content of tissues is decreased during a deficiency of this vitamin (*24*); correspondingly, Bellamy *et al.* (*2*) subsequently showed that the level of codecarboxylase (pyridoxal phosphate) in rat tissues was directly dependent upon the amount of pyridoxine fed (table 1). It is interesting to note that although 15 μg. of pyridoxine per day sufficed for maximum growth, higher intakes of the vitamin further increased the coenzyme content of the tissues. Similar data were obtained with a representative vitamin B_6-requiring bacterium, yeast, and fungus.

Commonly, a single coenzyme may be required for catalysis of many different reactions. For example, a recent review (*22*) lists 22 different enzymes that require coenzymes derived from nicotinamide, 14 that require coenzymes derived from riboflavin, and over 30 that require coenzymes derived from vitamin B_6. Any decrease in the coenzyme level of the tissues consequent to a deficient vitamin intake should thus reflect itself eventually in a lowered capacity of the tissue to carry out certain of the reactions dependent upon such coenzymes. Thus, a striking reduction in the ability of cells of *Streptococcus faecalis* to decarboxylate tyrosine (*8*) and to carry out certain transamination reactions (*17*) results when this organism is cultured in media low in or free of vitamin B_6 (table 2). It may be assumed that a similar lowering in the activities of unidentified essential enzymes in higher animals produces eventually a metabolic derangement resulting in production of the characteristic symptoms of vitamin deficiency.

TABLE 1

CODECARBOXYLASE (PYRIDOXAL PHOSPHATE) IN RAT MUSCLE IN RELATION TO PYRIDOXINE FED (*2*)

Pyridoxal fed	Codecarboxylase content of muscle
µg. per day	"Pyridoxal units" per mg. dry weight
0	0. 19
5	. 27
15	. 65
50	2. 6

TABLE 2

EFFECT OF VITAMIN B_6 CONCENTRATION ON THE TYROSINE DECARBOXYLASE AND GLUTAMIC-ASPARTIC TRANSAMINASE CONTENT OF STREPTOCOCCUS FAECALIS (*8, 17*)

Vitamin B_6 in medium	Activity of cells		
µg. per 10 ml.	Tyrosine decarboxylase	Transaminase	
	Q_{CO_2} (N)	Q_{trans} (N)	Q_{trans} (N)
0.0 [1]			0. 0
0.4 [2]	740	704	
4.0 [2]	1, 266		
40 [2]	2, 780		
100 [2]	3, 490	680	
3.0 [3]			64

[1] D-alanine added to an otherwise complete medium to permit growth in the absence of vitamin B_6 (*14*).
[2] Pyridoxine hydrochloride.
[3] Pyridoxal.

This description of the sequence of events in the production of a deficiency syndrome emphasizes a point tacitly assumed by most investigators, i. e., that an altered metabolism precedes clinically observable symptoms of vitamin deficiency. Why, then, are not tests of specific metabolic functions, e. g., the tissue levels of known vitamin-dependent enzyme systems, more widely used in the detection and study of subclinical vitamin deficiencies? Difficulties in obtaining appropriate experimental material aside, the answer to this question lies probably in two directions. First, such alterations in cases so far studied are usually quantitative rather than qualitative in nature; and suitable norms and the extent to which "normal" individuals may vary from these are not yet established. Second, with few exceptions, those enzymes so far examined have not shown readily measurable changes before other more readily observable deficiency symptoms appear. The reason for this may lie simply in observation of the wrong enzymes. Individual apoenzymes vary in the avidity with which they combine with their coenzymes; it may be expected, therefore, that as the coenzyme level of a tissue decreases due to vitamin deficiency, all of the coenzyme-dependent systems will not be affected equally. Those with high affinity for the coenzyme should remain maximally active, while those with lower affinity should decrease in activity. That this is true among the vitamin B_6-dependent enzymes can be seen by again referring to table 2. Cells of *Streptococcus faecalis* grown with high concentrations of vitamin B_6 (e. g., 40–100 γ of pyridoxine per 10 ml.) carry out transamination between glutamate and pyruvate at a rapid rate, and also actively decarboxylate tyrosine. At an appropriately reduced concentration of pyridoxine (e. g., 0.4–4.0 γ of pyridoxine per 10 ml.), but one that still suffices to permit excellent growth of the organism, the ability of the cells to decarboxylate tyrosine is greatly reduced, whereas the rate of transamination remains unaffected (*8*). Under these conditions the activity of another vitamin B_6 enzyme, the alanine racemase (*28*), also must remain at a level of activity fully adequate for growth, since synthesis of D-alanine is the growth-limiting reaction under these conditions (*14, 23*). However, if D-alanine is supplied preformed growth becomes possible without added vitamin B_6 (*14*), and the cells obtained cannot carry out the transamination reaction (*17*), racemize alanine (*28*), or decarboxylate tyrosine (*8, 16*). Addition of pyridoxal phosphate to dried cells obtained in this fashion restores all three of these activities.

Thus, one should not expect *all* of the enzymes dependent upon a given vitamin to decrease in activity during a deficiency of that vitamin of the degree that occurs naturally, or even that can be induced experimentally. Those enzymes requiring the highest concentration of coenzyme for activation should be affected first, and their reduced activity may suffice to slow growth or induce other deficiency symptoms before the majority of the enzymes dependent upon that same coenzyme show any reduction in their activities. Since we do not know all of the enzymes dependent for activity upon any of the coenzymes, those most sensitive to lack of these substances, and hence most useful for the detection of nutritional deficiency, may have escaped attention.

Only a few of the many known vitamin B_6-dependent enzymes have been followed during experimental vitamin B_6 deficiency in animals. Those that have been followed confirm the view developed above. Thus the level of cysteine desulfhydrase activity in livers of vitamin B_6-deficient rats decreases markedly to about 27 percent of the value in control animals within 3 days following withdrawal of the vitamin; within 7 days the activity had decreased to 13 percent of the value in control animals (*26*). Others (*12*, *19*) also have noted this rapid decrease in concentration of this enzyme; after 73 to 91 days on a vitamin B_6-deficient ration, Meister *et al.* (*19*) noted the complete absence of detectable levels of this enzyme in 6 of 10 rats tested. It appears probable that the level of cystathionase falls off at a similarly rapid rate during vitamin B_6 deficiency (*4*, *26*). In contrast, the glutamate-aspartate transaminase of rat heart muscle is reduced only to 66 and 40 percent of the control value after 2 and 6 weeks, respectively, on a vitamin B_6-deficient diet (*1*); and the glutamate-alanine transaminase activity of rat livers falls off at a similar comparatively slow rate (*19*). There is every reason to suppose (*20*, *22*) that the enzyme of rat liver cleaves threonine to glycine and acetaldehyde and is a pyridoxal phosphate enzyme. The activity of this enzyme, however, is not suppressed by experimental vitamin B_6 deficiency (*27*), indicating an affinity between coenzyme and apoenzyme such that the latter is saturated even at the lowered coenzyme concentrations in tissues of deficient rats.[1] [2]

I have emphasized these points because they appear to have been insufficiently recognized in the past. Relatively few studies of the relationship of vitamin intake to enzymatic function have been made; very few of these, in turn, have made any attempt to compare the *rates* at which various enzymatic activities fall off. For experimental nutrition, however, this is important information, because it is presumably those enzymes most rapidly affected whose hypofunction eventuates in the first observable symptoms of vitamin deficiency. If these were known, their determination might provide the means for detection of subclinical vitamin deficiencies and hence permit ready evaluation of the adequacy of vitamin intake. The sensitivity of enzymes concerned with sulfur metabolism to vitamin B_6 deficiency, described earlier, may explain the observed enhancement of symptoms of vitamin B_6 deficiency when high amounts of sulfur-containing amino acids are fed to rats (*6, 11, 26*). It suggests the feasibility of a "load test" for vitamin B_6 sufficiency in which the sulfate output following a test dose of cysteine is compared before and after administration of the vitamin. If the apoenzymes of man have similar affinities for their coenzymes as do those of rats [3], vitamin B_6-deficient individuals might be expected to form and hence excrete sulfate under these conditions more slowly than individuals in an optimum nutritional state.

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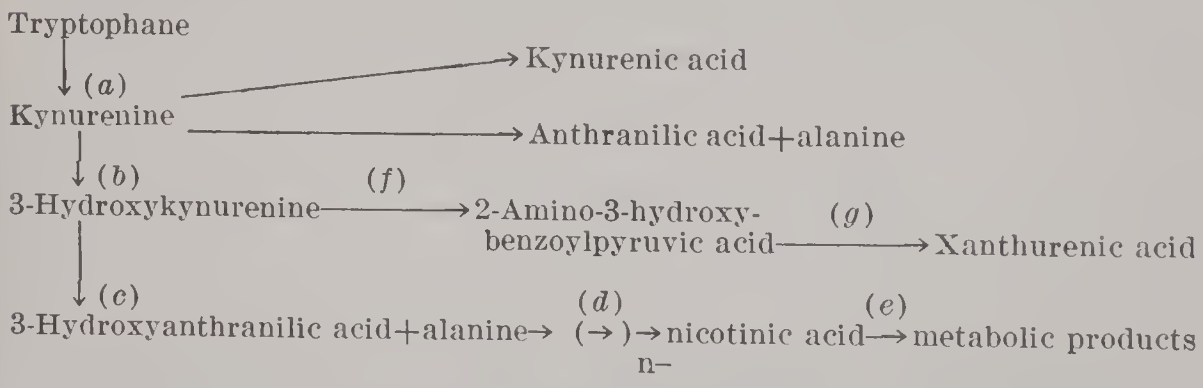
[1] Similarly, the glutamine-α-keto acid transaminase is not reduced in animals held from 73–90 days on a vitamin B_6-deficient diet (*19*). Whether this transaminase (like all others so far described) is a vitamin B_6 enzyme is not yet known; however, as Meister *et al.* (*19*) point out, the failure to observe decreases in its activity in vitamin B_6-deficient animals should not be considered evidence against this possibility.

[2] Just as the affinity of apoenzymes for their coenzymes vary, so should their affinity for various coenzyme analogies vary. There is, however, no reason for these affinities to parallel one another exactly. For this reason, a vitamin deficiency induced by feeding a vitamin analogue need not simulate exactly that induced by deprivation of the vitamin since the enzyme deficiencies leading to symptoms may differ in the two cases (cf. (*12*)).

Thus once those enzymes that are unusually sensitive to vitamin deficiency are discovered, a rational basis for devising suitable load tests, and by-passing the necessity for tissue samples, should be at hand. For present load tests represent nothing more than differences in metabolic capacities of experimental and control subjects, and such differences obviously must have an enzymatic basis. The test load serves to magnify the observable difference by more nearly saturating the enzymes concerned with substrate, thus causing them to work at more nearly their capacity. Current tests of this nature used experimentally in suspected vitamin B_6 deficiency consist in administration of a test load of alanine or of tryptophane. With the former, an unusually high level of blood urea results in vitamin B_6-deficient as compared to normal (or vitamin-supplemented) subjects (*18*). The enzymatic basis for this difference is unknown. In the tryptophane load test, a test dose of tryptophane results in enhanced excretion of xanthurenic acid by deficient subjects as opposed to normal controls (*13*). This result can be rationalized by assuming that the excess of tryptophane normally is metabolized in part *via* reactions (*a*) through (*e*) below.



The enzyme kynureninase, effecting reaction (*c*) is a pyridoxal phosphate enzyme, and has been shown to occur in reduced amount in vitamin B_6 deficiency (*5, 10*). Reaction (*c*) is thus slowed, and 3-hydroxykynurenine may accumulate, its increased concentration forcing metabolism through reactions (*f*) and (*g*), thus leading to excretion of xanthurenic acid. Reaction (*f*) also is catalyzed by a vitamin B_6-

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[3] Since the corresponding proteins of different species are immunologically distinct and hence vary measurably in structure, there is no reason why this must be so; nevertheless, high probability of close similarity would exist.

dependent transaminase (*9*, *10*), but this does not invalidate the proposed explanation if this enzyme has a higher affinity for pyridoxal phosphate than kynureninase, or if it occurs at a substantially higher concentration. The excretion of many of these intermediates of tryptophane metabolism by vitamin B_6-deficient rats has been demonstrated by Dalgleisch (*9*).

Reference was made earlier to the fact that a high intake of a vitamin, specifically vitamin B_6, may produce a higher coenzyme content of tissues than occurs on an excellent stock ration. Whether such coenzyme levels are advantageous, or whether they can on occasion be harmful to animal life is not known. In bacteria, where lack of control by the organism makes extreme variation and rigorous control of the environment possible, conditions can be arranged such that either effect is observed. For example, in media containing marginal amounts of tyrosine, organisms richly provided with vitamin B_6 will not grow as heavily as organisms grown in the absence of this vitamin (*16*). This effect is shown in table 3, which also shows that under these conditions vitamin B_6 is not markedly deleterious to growth provided tyrosine is supplied in peptide rather than in free form. The explanation for the deleterious effects of vitamin B_6 here lies in its activation of the tyrosine decarboxylase which, by converting part of the limited supply of free tyrosine to tyramine, decreases the supply of this essential amino acid below that required for maximum cell formation (*16*). If, however, tyrosine is present in sufficient excess, or is supplied in peptide form, then a high concentration of vitamin B_6 has no deleterious effect on growth. With free tyrosine in excess, if the environment were modified in a way that intensified the requirement of this organism for carbon dioxide (e. g., by decreasing the pH of the medium and employing a small inoculum), the high level of vitamin B_6 should actually favor growth,

TABLE 3

THE EFFECT OF VITAMIN B_6 ON STREPTOCOCCUS FAECALIS IN THE PRESENCE OF VARIOUS AMOUNTS OF TYROSINE OR TYROSINE PEPTIDES (*16*)

L-Tyrosine (μM per 10 ml.)	L-leucyl-L-Tyrosine (μM per 10 ml.)	Incident light transmitted, percent [1]	
		No vitamin B_6	With pyridoxamine [2]
0	0	94	96
0.4	0	57	89
1.2	0	40	82
5.4	0	40	49
0	0.4	53	67
0	1.2	40	45
0	5.4	------	40

[1] 100 percent incident light transmitted indicates no growth.

[2] 100 μg. per ml. of medium.

since production of carbon dioxide by decarboxylation of part of the tyrosine would then be favored (*16*). This, then, constitutes a striking demonstration of the dependence of the requirement for one nutrient upon the level of others present. Thus with vitamin-requiring bacteria, there is no such thing as an optimum "intake" of the vitamin that can be specified apart from the ration of which it is one component. One may at least suspect that a similar situation will be found to hold in animals.

Comparatively few examples of the above nature, where a high intake of a given nutrient can be harmful, innocuous, or helpful de-depending upon the environment, have been described. Much more common are those cases where the vitamin requirement may vary greatly on rations of different composition, but amounts over and above that necessary for maximum growth are harmless. An extreme example of this type, again taken from bacterial experiments, is shown in table 4. The vitamin B$_6$ required for half-maximum growth of *Streptococcus faecalis* varies from zero on a medium complete with respect to known physiologically important amino acids to over 100 mγ of pyridoxamine per 10 ml. in a medium in which α-hydroxyisocaproic acid is substituted for the amino acid, leucine (*15*).

TABLE 4

COMPARATIVE VITAMIN B$_6$ REQUIREMENT OF STREPTOCOCCUS FAECALIS FOR GROWTH WITH AMINO, KETO, OR HYDROXY ACIDS (*15*)

Acid corresponding to	Additions to appropriate deficient medium [1]		
	Amino acid	Keto acid	Hydroxy acid
	Pyridoxamine required for half-maximum growth, m μg. per 6 ml.		
Histidine	0	5.7	[2]
Isoleucine	0	8.8	100
Leucine	0	2.7	[2]
Tryptophane	0	17	[2]
Tyrosine	0	8.2	[2]
Valine	0	4.7	60

[1] Nutritionally adequate except for the metabolite indicated and vitamin B$_6$.

[2] These hydroxy acids cannot replace the corresponding amino acids for growth even in the presence of 10 μg. of pyridoxamine per 6 ml.

It may be assumed that variations similar in nature to these, although perhaps of smaller magnitude due to the greater constancy of the nutritional and internal environment, occur in the requirements of animals for various vitamins. Indeed, the vitamin B$_6$ requirement of rats is known to increase as the protein content of the ration is increased (*21*), and to vary with the content of the ration in specific amino acids (*7*, *11*). The magnitude of the variation that can exist has not been determined. Because the magnitude of the requirement for a vitamin may differ on different regimens, and because during a suboptimal vitamin intake the vitamin-dependent reaction that limits physiological well-being may vary similarly, it may be overoptimistic to believe that any single test of a specific metabolic function will suffice to indicate whether or not the vitamin intake of an individual is sufficient.

In summary, we have seen that the rates at which specific vitamin-dependent enzymatic reactions decrease during vitamin deficiency varies markedly from one reaction to another, and that this is a consequence of the differing affinities of the individual apoenzymes for their common coenzyme. Insufficient investigation of such rates has been made, and, in general, we do not know which enzymatic reactions are most sensitive to vitamin lack. When this information becomes available a rational basis for devising really sensitive load tests for evaluation of nutritional adequacy will be available. Such load tests represent in essence simply an overloading of one or more enzymes with substrate, thus causing these to work at their maximum rate, and emphasizing any differences that exist between experimental and control subjects.

It appears from current knowledge that there is no single "optimum" intake of a vitamin, but that the optimum amount may vary significantly from one regimen to another. Because of this, it may be that no single test for adequacy of intake of a given vitamin can be devised.

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Discussion

CHAIRMAN NELSON

Dr. Bessey, you mentioned the fact that there are a number of conditions in which the vitamin A of the blood serum may be low due, for example, to infections. You said in most cases you could determine that this was due to some abnormal condition. How can you determine that? What guidelines do you have?

BESSEY

The guidelines are the same, in my opinion, as the guidelines the physician uses for eliminating a second possibility when he is considering any type of pathological situation. This sounds like evading your question. I suppose that is the case, but the only reported cases of vitamin A being low in the blood due to conditions other than vitamin A deficiency have been infectious processes that would cause no trouble in recognition.

That is the only guide, and there is no real certainty. You have to work on the basis of the odds being very great. Let's say if you examine a population with respect to this and find 20 percent with vitamin A below this accepted figure, it isn't likely that you are going to have 20 percent of these people with infectious processes without their being aware of it. But, I think there is no way of guaranteeing that this is the case. I think it is true of both vitamins A and C as well as the other vitamins. Any time the levels drop below a given point, it is presumptive evidence that you may have trouble. It is not certain that you may have vitamin A or vitamin C deficiency in the case of the lack of vitamin A or C, but the chances are good.

SEBRELL

One of the things that came out of the nutritional pregnancy study was that we had failed to recognize normalcy or normal psychological processes in the past.

Under normal circumstances, we feel there are hydration phenomena in pregnancy, and we have assumed that the normal hemoglobin level, to distinguish between the anemic and the nonanemic, has 10 g. of hemoglobin. In pregnancy when a peak hydration is present, that lower limit of normal must be dropped. We didn't like the idea. It took us by surprise. But it drops down to about 9.3 hemoglobin and still maintains a normal picture. The reverse is true of urine carotene and urinary excretion of uropepsin. It goes up and reverts to normal postpartum and I think with all of these we will make a plea for recognition of an infectious status so far as the whole problem is concerned.

CARTWRIGHT

With respect to the evaluation of blood level in a normal subject, I think one of the biggest problems occurs when infection or fever is present. A whole multiplicity of changes take place. The cell contents in the blood are modified by infection.

Two or three of the vitamins have been studied, including vitamin A, and it has been shown that infection will markedly modify the vitamin A level. Sufficient studies have not been performed with patients with extremely mild infections such as common colds and pharyngitis to determine what influence these have on plasma level. Those substances which have been studied in the blood have been shown to be altered markedly by even such small infections as the common cold. Therefore, going into any population group would necessitate a complete medical history and physical examination, especially with regard to diseases suffered in the several weeks prior to the survey.

OSSERMAN

Is there evidence to show that the serum ascorbic acid level is significantly depressed by an acute alarm or acute stress situation?

BESSEY

The evidence on the serum ascorbic acid is not very clear. There are reports that it does decrease and others that it does not. I think it is fairly clear that under continued stress conditions ascorbic acid disappears somewhere. I am referring to the work done in the studies on burns in Boston which showed that you must give very much larger amounts of ascorbic acid in order to get equivalent excretions under chronic stress such as a burn. However, in most cases, the blood level was down.

I don't know from personal experience, but there are conflicting reports in the literature as to whether blood levels always go down as a result of acute stress. It goes down in the adrenal glands, but it isn't certain that this means the asorbic acid is used up. It just means it disappears from the adrenals. However, it doesn't go down in the liver or certain other tissues in the animal in acute stresses as in the adrenals. It may be a transfer out of the adrenals.

The requirements for ascorbic acid, based on using blood levels, seem to be increased in acute stresses. It is not clear.

ROBERT M. KARK (University of Illinois)

We have studied the stresses and the adrenal gland in man. There is a rise of ascorbic acid in serum. Under situations where you have distress of surgery, as shown in work done by Dr. Emerson here, where it is a fault of ascorbic acid, that can be blocked by stimulating the adrenal gland with ACTH, and instead of having complete drop, you will get a bit of a rise.

OSSERMAN

It is indicated that the ascorbic acid is not being utilized for steroid production.

KARK

The adrenal ascorbic acid has nothing to do with steroids.

OSSERMAN

The small carbon fragments of the ascorbic acid molecule can be used for steroid production.

KARK

Certainly, but how much is used doesn't matter altogether. As I say, when you have gross surgery, you can stimulate the adrenal glands and they will react pretty much in the normal way.

M. K. HORWITT (Elgin State Hospital)

In reporting the results of long-term analysis under conditions where you are trying to evaluate nutritional adequacy, and eliminating those conditions of stress which might come from burns and from surgery, and only considering the intercurrent illnesses that come in the course of a 3-year observation in our studies, we did not observe any changes due to cold or mild infections that would have affected our interpretation—on ascorbic acid, riboflavin, and nicotinic acid.

SPECTOR

Dr. Unglaub might be able to answer this. We would like to hear what experience has been had with the test, recommended by Dr. Snell, of loading up the body with a certain amount of tryptophane and then measuring the excretion of xanthurenic acid in the urine as a measure of pyridoxine deficiency.

UNGLAUB

We discovered that would be covered more clinically under metabolic studies. As you know, the tryptophane load test to estimate pyridoxine deficiency is reported by Vilter in his studies with pyridoxine. He has found that the excretion of xanthurenic acid following such load tests is not as reliable an index in adults as in children. On the other hand, these studies, reported by Dr. McGanity in more detail, show there are xanthurenic acid excretions during pregnancy. How many of these studies have been done and correlated with given levels of intake is another matter.

Pyridoxine deficiency has not been observed under normal circumstances, and I think when these things are interpreted you have to take into consideration the possible effects of any antagonist that is used outside of a simple displacement of the vitamin involved. You have to take into consideration that these antagonists may have specific effects of their own.

McGANITY

Worthstein and his group were responsible for the only serious criticism that can be leveled on basic interpretations of the pregnancy studies, and that is that the period of time during the study—the immediate 24 to 48 hours following delivery—is the period in which the most rapid changes in electrolyte and fluid balance take place. Our feeling is that it should be repeated on a trimester basis or following a series of 10 cases.

JOLLIFFE

I would like to ask Dr. Unglaub a question. There was no correlation between the urinary excretion and the clinical signs of riboflavin in the Newfoundland study?

UNGLAUB

That is right.

JOLLIFFE

The record up there shows a correlation at a level of less than 0.01 for at least three clinical signs according to the statistical analysis.

UNGLAUB

I believe the statement was made by the groups who reported that study that they were unable to make any definite correlation.

JOLLIFFE

I just went over it for my paper and that is what it showed—that they reported a statistical significance with a probability of less than 0.01.

CHAIRMAN NELSON

Any further questions or comments?

E. ALPERT (Merck and Co.)

I would like to point out that there are becoming available methods for determining B_{12} absorption and excretion through the use of radioactive B_{12}. There are at least three such methods; one a measurement of urine excretion, one of radioactivity of stools, and the third, an assimilation counter of absorbed radioactive B_{12} by the liver.

BESSEY

On this point about there being other conditions, pathological and otherwise, that may influence remaining riboflavin, I think we should remember it is no different from all the other chemical tests we use in pathological situations. We don't say because blood sugar is up that this is a guarantee that the patient has diabetes, because blood sugar level can be raised by a number of things. It means we will look into it a little more carefuly to see if it is diabetes or something else. You don't say because urea nitrogen is up, the patient necessarily has something wrong with his kidneys—there can be various reasons. While it is worth investigating to see what the trouble is, we must not magnify the possible difficulties from this other source, but rather must keep it in mind when we are evaluating our data.

SEBRELL

I think there was a note of skepticism in Dr. McGanity's report about magenta tongue. Lest someone have the idea that such reported lesions are merely kodachrome defects, I would like to say there is such a lesion as magenta tongue as clearly established by clinical observation. It was first seen in Dr. Sydenstricker's clinic in Atlanta, Georgia, and I believe he saw a lesion. This was further demonstrated by Dr. Julian Ruffen of Duke University, who was with us for a while in Germany and who didn't believe there was any such thing as a magenta tongue until he saw so many of them in the German children.

There is such a lesion. Whether or not it is due to riboflavin deficiency may be subject to debate. I don't believe the reason for the actual cause is clearly established. Among other things that perhaps should be cleared up for the record, the lesion that occurs in the angles of the mouth, known as angular stomatitis or by other names, and which Dr. McGanity has mentioned as sometimes seen in ill-fitting dentures and various other conditions, is a symptom which is due to a variety of causes, just as polyneuritis may be due to a variety of causes. You still have to make a diagnosis after you see the lesion, and one of these diagnoses, I believe, is riboflavin deficiency. There are a number of other conditions that cause this lesion and these have to be differentiated.

I also feel that the audience should not be left with the impression that the answer to peridontal disease is merely good mouth hygiene. The dentists I am familiar with feel that the cause is unknown. Much research is necessary before we understand all the mechanisms involved in peridontal disease.

L. B. PETT (Dept. of National Health and Welfare, Ottawa)

I would like to cite the statistical paper presented by Dr. Crampton in reference to some of the problems of blood values that have come up in all the papers. Namely, when you are dealing with means you often get a wide deviation of results, and when you compare the mean of one population with the mean of another population, you are faced with a question of whether it is true. As Dr. Bessey said—there is a difference, but it is not a significant difference.

The idea suggested by Dr. Crampton is actually a suggestion that would permit a more accurate evaluation of the place of an individual rather than the mean of the population.

IV. Evaluation of Mineral Adequacy

Balance Studies With Macro-Elements

D. M. Hegsted

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School of Public Health [1]
Harvard University

As I interpret my assignment today, it is to evaluate the balance method as applied to the so-called macro-elements—calcium, phosphorus, magnesium, sodium, and potassium. The extent of our knowledge about nutritional requirements of these various minerals varies a great deal. Essentially, everything recorded relative to adult requirements for calcium, phosphorus, and magnesium has been obtained with the balance method. Our knowledge of sodium and potassium comes to a considerable extent from clinical experience. I am concerned primarily with the balance method and its shortcomings or advantages in evaluating nutritional requirements or the adequacy of rations. I shall speak principally of calcium because our data for this mineral are more extensive than for other minerals, and it has often been said that calcium is the nutrient most often lacking in common diets, but I believe that in general the comments apply to other minerals as well.

In 1941 Dr. Sherman (*14*) stated the general conclusion at that time in his well-known text, "The method which permits the most direct approach to the question of the amount of calcium or phosphorus needed in human nutrition is that of the balance experiment." Shohl (*15*) had pointed out, however, that "This method can never give more than a bank balance; it cannot state how the income was invested but only how much was spent."

This criticism does not include the most serious fault of the balance method. One would like to know where and how the calcium, for example, is distributed in the body under various conditions, but we may perhaps expect that if sufficient is provided, the body will utilize it and distribute it satisfactorily. The fault of not telling us how the income was invested may not be too serious when we are concerned with requirements.

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[1] Studies on calcium metabolism in this department have been supported by research grant A–194C from the National Institute of Arthritis and Metabolic Diseases.

The real trouble with the balance method which has not been, and is still not generally appreciated, is that it does not tell us how large our bank balance is. It tells us what we put in and take out but not the size of the balance. Most of us like to know the size of our bank balance. In a lean period we may want to draw on it more heavily than usual. We want to know how much we can take out and what the consequences may be. On the other end of the scale, we like to know how large our account is. If it should get too large, I am told that one may wish to invest the funds in other activities that may be more useful or profitable, rather than simply hold them in the checking account.

The balance method applied to nutrition studies usually presents the same problems. Not knowing where we start, it tells us whether or not we are keeping even, gaining, or losing. To justify the use of the balance method as a measure of requirement or adequacy of the diet, one must make a supposition at the beginning that may have little real meaning. This supposition is usually stated in the paper as, "The studies were done upon x number or normal healthy individuals." This statement means a great deal, of course, but at the same time there are many things it doesn't tell us.

How little it may mean in terms of calcium requirements is evident when one compares studies done on subjects accustomed or adapted to different calcium intakes.

We studied the amount of calcium required to maintain balance in Peru (*5*) on "normal" adults in the penitentiary. These subjects had essentially no milk in their diet and had had low-calcium intakes for many years. About 0.2 to 0.3 gm. per day were required on the average for balance.

Similar studies done principally in this country on "normal" adults give us values of about 0.5 (*13*) to 0.7 (*10*) or even higher. It is more surprising perhaps that the response to added calcium seems to be about the same (fig. 1). This is seen by the nearly parallel slopes of the regression lines correlating intake and excretion obtained by us and by Mitchell and Curzon (*10*). As the intake is raised, positive balance is produced. The degree of positive balance obtained for an increase from the intersection is essentially the same for the two lines. This seems particularly significant to me because I feel that it is a biological law that *the body makes more efficient utilization of a nutrient when the body is in need.* The people accustomed to the low-calcium diets apparently do not retain added calcium with any more efficiency than those adapted to higher intakes.

One can approach the problem of balances from an entirely logical point of view rather than a study of the experimental data. By this I mean simply that *all so-called normal adults* whether they are found eating low-calcium diets or high-calcium diets *are in balance.* Nutritionists have traditionally looked with horror on negative balances

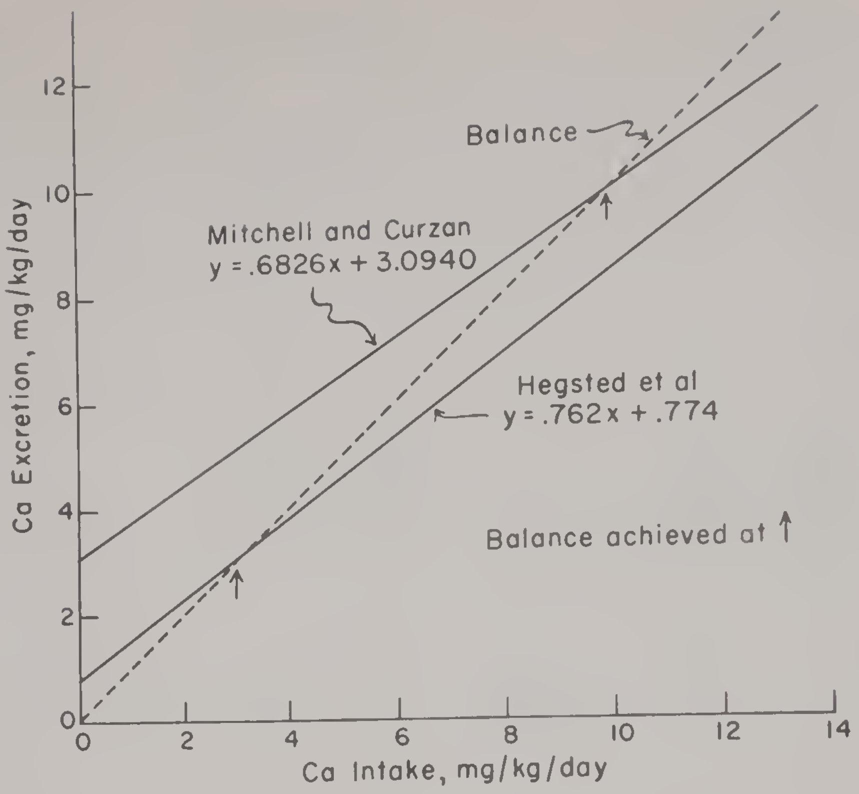

FIGURE 1.—The relation between calcium excretion and intake in the data summarized by Mitchell and Curzon (*10*) and those obtained by Hegsted *et al.* (*5*). The regression lines are essentially parallel.

and with favor on positive balances. Now from the long-range point of view it is evident that neither a continued positive balance or a negative balance is desirable. If the former is continued, the adult will end up as a block of calcium phosphate. If a negative balance is continued, he will end up without any bones. Neither of these is generally found. Thus adults in general are in balance. The studies of Cobb (*1*) suggest that calcium retention may continue until age 30 or so, and that losses of calcium may be common after age 65. Whether this is true or not we do not know. If it is, we have no indication as yet as to whether it is desirable or undesirable in terms of health nor whether calcium intakes would exert more than a temporary effect.

One may reach the conclusion upon theoretical grounds that the amount of a nutrient required to maintain balance in a "normal adult" is that amount which his traditional diet supplies.

The simplest explanation of the adaption to different intakes would be to compare the body to a leaky bucket. With obvious over-simplification, one may consider the diagrams in figure 2. The arrows at the top indicate the amount of intake and the arrows at the sides the excretion. If one pours in 0.8 gm. of calcium per day in an "adapted" subject, the output is also 0.8 gm. The same is true of the subject adapted to 0.4 gm. per day. Now if we increase the intake of both buckets by 0.2 gm. per day, the level will gradually increase for a period and both will be in positive balance. Finally after the adjustment period the output will eventually reach the intake and both will then be in balance.

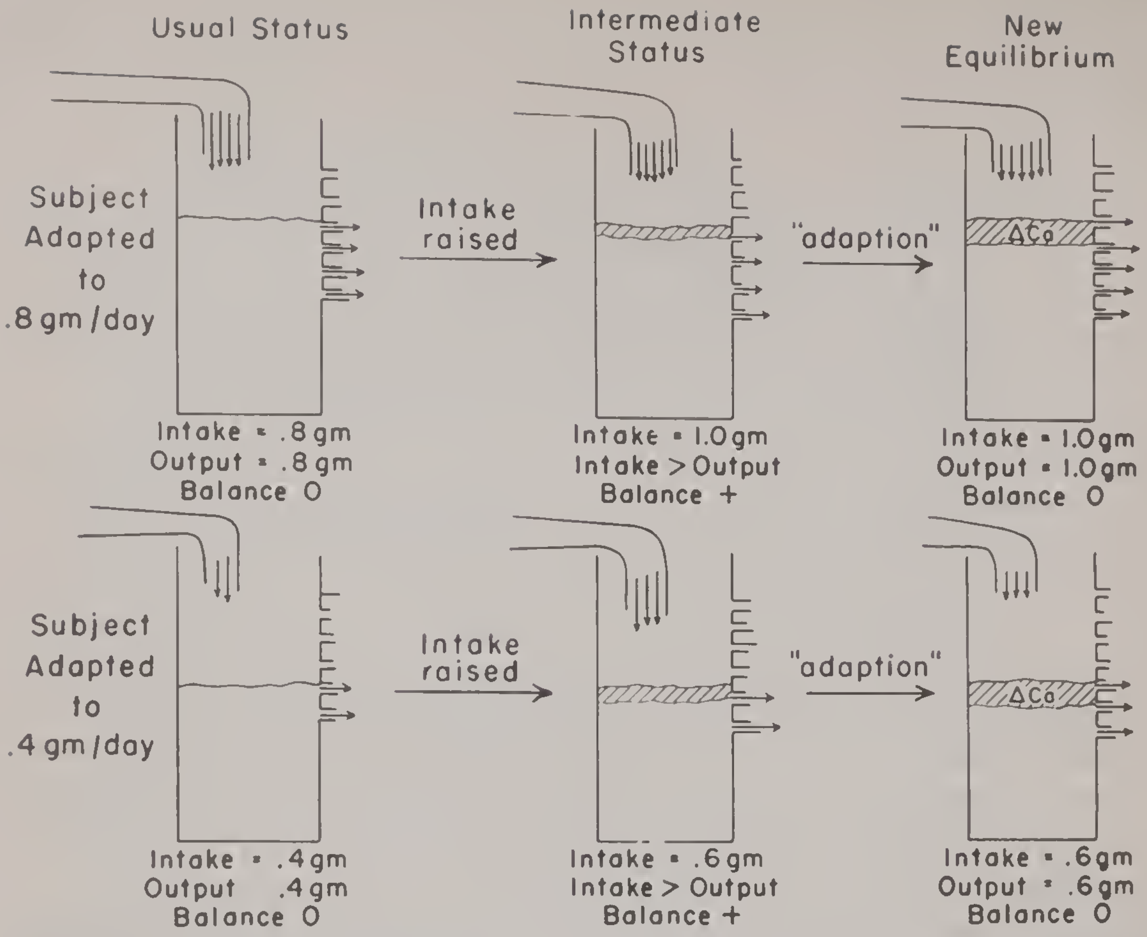
Usual Status
Intermediate Status
New Equilibrium
Subject Adapted to .8 gm/day
Intake raised
"adaption"
ΔCa
Intake = .8 gm
Output = .8 gm
Balance 0
Intake = 1.0 gm
Intake > Output
Balance +
Intake = 1.0 gm
Output = 1.0 gm
Balance 0
Subject Adapted to .4 gm/day
Intake raised
"adaption"
ΔCa
Intake = .4 gm
Output .4 gm
Balance 0
Intake = .6 gm
Intake > Output
Balance +
Intake = .6 gm
Output = .6 gm
Balance 0

FIGURE 2.—Simplified scheme to demonstrate balance at various levels of intake on subjects accustomed to different intakes and the "adaption" to a change in intake.

Now it must be recognized that we know essentially nothing about the size of the increment in calcium, ΔCa. Carrying our analogy a little further, the effects may be quite different depending upon the shape of the bucket (fig. 3). The body content may change much or little after a dietary change. It might be the same or very different in different subjects. Of course, the size of ΔCa is not so important as any effect it may have upon health. Of this we know practically nothing.

I hope no one will take these diagrams very seriously. While we know that raising the intake will increase body stores at least somewhat, we also know that there are many homeostatic mechanisms which the diagrams ignore. Certainly the body calcium is not proportional to intake. People from populations adapted to low-calcium intakes are usually of smaller stature, cause unknown, but the skeletal size is certainly not proportional to calcium intake. Endogenous factors characteristic of the individual control the kidney excretion to a large extent (*8*, *11*). It is possible that these may be related to past dietary habits.

In this regard the recent work of Henry and Kon (*6*) is significant. These workers fed rats different levels of calcium from weaning to approximately 2 years of age. The animals on the low level grew

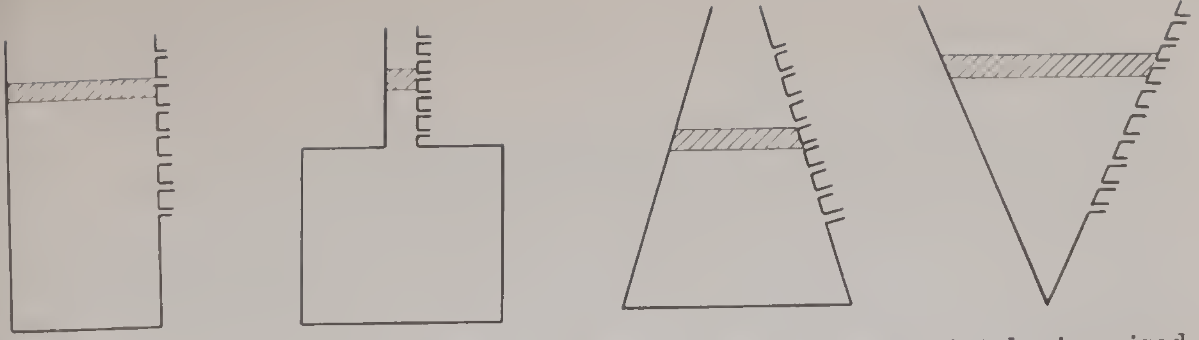

Figure 3.—The amount of material gained by the body as the intake is raised may be variable, the same, large or small. This is unknown at present.

somewhat more slowly and bone ash was somewhat lower for a time, but eventually they caught up to those on the higher calcium level. Throughout life and even in old age, however, they retained calcium better than the high-calcium group when tested upon a low-calcium diet. The authors suggested that perhaps with some degree of calcium restriction, the bone salt is deposited in a form which exchanges less rapidly. It leads one to speculate that perhaps when high levels of calcium are fed over long periods the "calcium retaining mechanisms," whatever these may be, might have little work to do and become atrophied. One can think of many instances, of which the musculature is the most obvious, where exercise of the function is necessary to keep it operating efficiently. It is well substantiated that negative calcium balances are common in old age. The traditional view is that we should load up so that we will be well prepared when this time comes. It is possible that this logic is completely false. We should recognize the fact that we do not know.

With regard to magnesium the summaries of Duckworth (*2*, *3*) are of interest. On the basis of balance studies he estimated the magnesium requirement to be about 250 mg. per day. The actual intakes in England were found to be related to the economic level or the expenditure for foods. The two lower income groups evaluated did not reach the estimated requirement. As is clear from the discussion so far, I doubt that this approach is useful. However, the reason for pointing these findings out is simply that the approach to requirement and estimation of the extent of deficiency is exactly the same as has been used for calcium. Based upon the data available we should be as worried about magnesium as we are about calcium.

Very little is known about the requirements of sodium and potassium. There appears to be no reason to suspect deficiencies in usual diets consumed by human beings. Deficiencies of potassium are now recognized as not uncommon in certain clinical conditions (*7*). The maintenance requirement of sodium is known to be low as shown by studies upon the low-salt diets (*7*). We should note that these levels of sodium are much lower than are required to maintain balance in the average individual and that important adaptive mechanisms come into play to conserve sodium when losses in the sweat are high.

It will also be recognized that it takes some time to reach the adapted state.

Before concluding we must point out that balance does not mean that the output and intake are equal all the time. Whether a subject is in balance during any particular period depends primarily upon the accuracy of the study. Since diets vary and other physiological and environmental factors vary, balance means only that the positive and negative values are equal. One can only expect therefore what is found in practice (*4*, *12*), that in a group consuming their usual diets, approximately half will be in negative balance and the other half in positive balance. The common conclusion, that the half of the population found to be in negative balance of the nutrient under study is underfed with respect to that nutrient, simply is not valid.

One reaches the unfortunate conclusion therefore that we simply are ignorant of the calcium, phosphorus, and magnesium requirements of the adult. Our estimated requirement of calcium of around 0.7 gm. per day simply reflects the dietary habit of the population upon which the studies were done. Had we worked in areas where milk and other calcium containing foods were less common, we would have arrived at estimates of perhaps half as high. It seems apparent that if we continue to apply the logic we have been using, our estimates of requirement will increase as the intake increases with no end in sight. We are desperately in need of other methods of determining requirements of these minerals and their effects upon health. It would appear likely that studies done upon groups consuming low diets will more probably give us the leads we need than when the diets are high.

Most of us have told our students about the Voit protein standard. It seems that Voit determined the intake of the Bavarian farmers, thought that they were healthy, and therefore decided that this ought to be a reasonable standard. To give the proper impression, the story is told with a supercilious smile to indicate how naive they were in years past. Well, we certainly devised more complicated methods. How much more we can say than this is open to debate.

I do not wish to leave the impression that the balance method has no value. It may often be the method of choice when one is studying the effect of some condition or dietary modification upon the metabolism of a nutrient. The response of a subject to increasing levels of a nutrient may be informative. The change in balance of one nutrient by modifications of another, calcium balance as affected by phosphorus intakes or citric acid intakes, for example, is worthy of study. In fact one might conclude that whenever we are studying a change in status, the balance method may be useful. How useful it will be may depend primarily upon the response of the organism to the change, the rapidity and extent of change thus induced, in relation to the facilities for study.

If we have good reason for believing that the subject under study is optimally nourished when we begin the work, a balance study will certainly tell us whether we are maintaining the supply or not. We could probably all agree that diets which cause large losses of a nutrient from the body should be looked at with suspicion. The final conclusion as to the advantage or disadvantage of the situation in terms of health will depend primarily upon how effective the adaptive mechanisms of the body are. These have been relatively little studied and require a great deal of work. The studies of Nicolaysen on calcium (*11*), the extensive work of McCay (*9*) on restricted diets, and the recent work of Henry and Kon (*6*) provide excellent examples in this direction.

Finally, it is probably true that a great deal of useful work could be done upon subjects adapted to what we ordinarily consider poor diets. In most nutrition studies with experimental animals or human beings the most useful approach has been to study the deficient organism. This gives us leads to what to look for, criteria of deficiency, etc. For calcium, phosphorus, magnesium, potassium, and, to a lesser extent, sodium, most of our present-day information has not been gained in this manner. Rather we have studied primarily reasonably well-fed populations. More studies upon people consuming limited diets should be very useful.

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Specific Functions of Trace Elements in Blood Formation

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Of the "trace elements," iron, copper, cobalt, and zinc are definitely known to be involved in hemopoiesis (*3*). As yet, insufficient knowledge has accumulated regarding manganese, magnesium, molybdenum, and other minerals to make it possible to assess their role in blood formation in man.

Evaluation of the nutritional adequacy of iron is of considerable practical importance. Although it is conceivable that under special circumstances copper depletion may exist in man, to date this has not been demonstrated conclusively. It is unlikely that cobalt, zinc, manganese, molybdenum, and other trace elements would be of any importance from the standpoint of dietary inadequacy. In any event, studies to date are insufficient to permit one to draw any definite conclusions.

Iron

Before discussing methods for evaluating iron adequacy it is important to make clear that there are two forms of iron deficiency, latent and manifest (table 1). In latent iron deficiency the available iron stores are depleted, the plasma iron level is reduced, and the iron-binding capacity of the plasma is increased. However, no anemia is present. In manifest iron deficiency the available stores are depleted, there is hypoferremia, an increase in the iron-binding capacity and in varying degrees the characteristic microcytic, hypochromic anemia is present. Methods for evaluating iron adequacy must, therefore, include procedures for detecting the adequacy of the available iron stores (latent iron deficiency) as well as the manifest deficiency state.

TABLE 1

Latent Iron Deficiency

1. Hypoferremia.
2. Increase in total iron-binding capacity.
3. Depletion of tissue stores.

Manifest Iron Deficiency

1. Hypoferremia.
2. Increase in total iron-binding capacity.
3. Depletion of tissue stores.
4. Microcytic, hypochromic anemia.

As the dietary intake of iron falls below the demand for iron, contraction of the available iron stores is probably the first change which occurs. After the available stores become low, there is a decline in the plasma iron level from the normal value of 110 μg./100 ml. to the low level of 10 to 30 μg./100 ml. Concomitant with this, the concentration of the iron transporting protein (transferrin) in the plasma increases with the result that the iron-binding capacity of the plasma rises from the normal of 300 to 350 μg./100 ml. to over 400 μg./100 ml. After the available iron stores have become depleted, anemia supervenes.

An analysis of the methods (table 2) of appraising the nutritional state of the human subject in reference to iron will be discussed separately.

TABLE 2

METHODS FOR THE EVALUATION OF IRON ADEQUACY

1. Plasma iron level.
2. Total iron-binding capacity of the plasma.
3. Tissue iron (bone marrow hemosiderin).
4. Volume of packed red cells.
5. Corpuscular constants.
    a. Mean corpuscular volume.
    b. Mean corpuscular hemoglobin concentration.
6. Examination of the peripheral blood smear.

Available iron stores.—A simple method for the estimation of the iron stores in man has been described by Rath and Finch (*25, 26*). An unstained smear of bone marrow aspirated from the sternum is examined for hemosiderin and the iron content of the marrow smears is graded from 0 to 6 plus. It has been shown that the data obtained by this technique are reliable as an index of iron stores in man. For example, in 31 normal subjects the amount of iron present was graded as 0 to trace in 4, 1 to 2 plus in 21, and 3 to 4 plus in 6. In uncomplicated iron deficiency the amount of iron present was 0 to trace in 54 patients and 1 to 2 plus in only 4 patients. More than 2 plus iron was found in none of the iron-deficient patients. Furthermore, it was possible to demonstrate a difference in the size of the iron stores between the males and the females even though neither sex was clinically iron deficient. The lower stores in the female presumably reflect the iron losses during menstruation and pregnancy. In the normal subjects with no hemosiderin demonstrable in the marrow and no anemia or hypoferremia, removal of iron by phlebotomies was followed by the rapid development of anemia whereas this did not occur as soon in those in whom hemosiderin was demonstrable in the marrow.

Plasma iron level.—Hypoferremia is present in all patients with manifest iron deficiency (table 3) and in most patients with an appreciable contraction of the iron store. Unfortunately, however, hypoferremia is not specific for iron deficiency and also occurs in patients

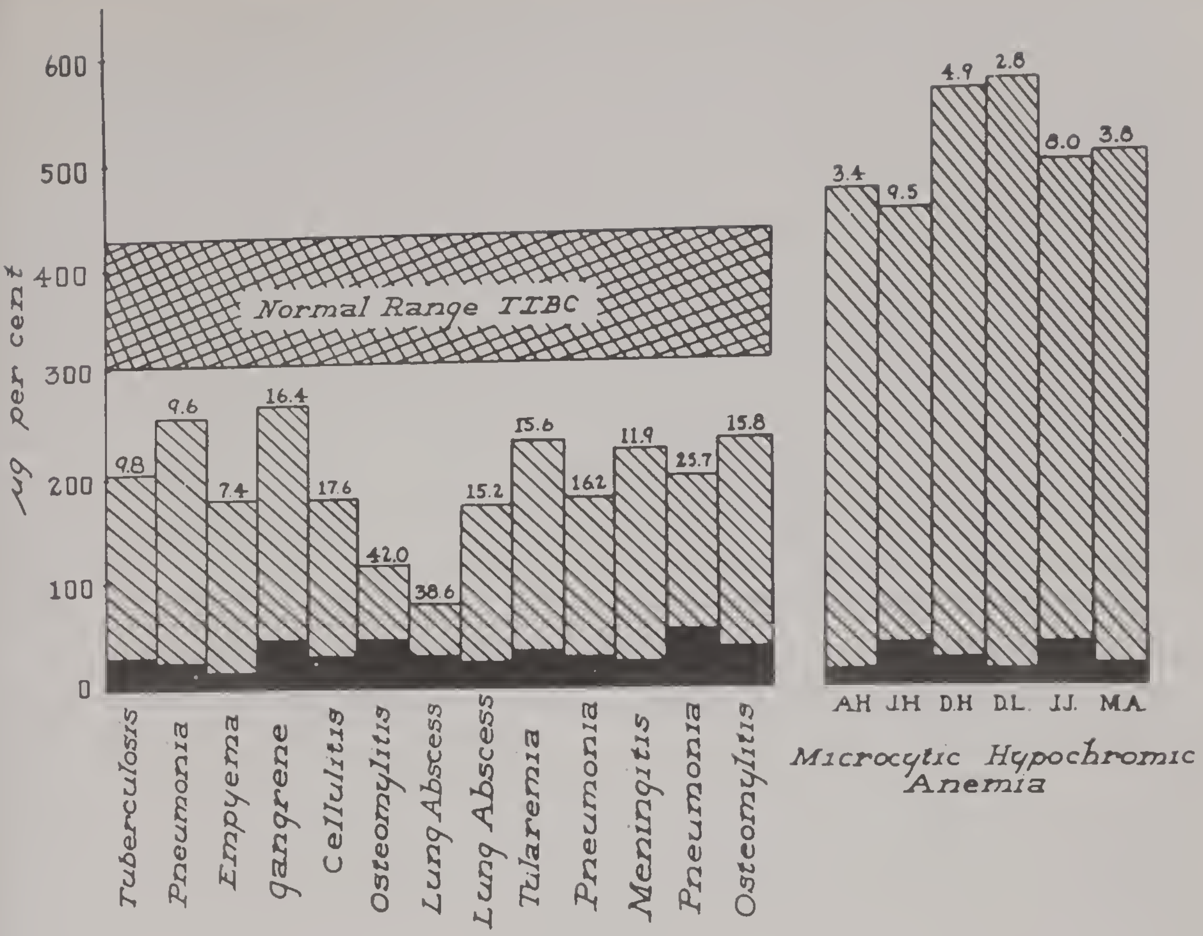

FIGURE 1.—The total iron-binding capacity of the serum in patients with iron deficiency anemia as compared with the normal and patients with anemia associated with chronic infection.

with infections (*8*). Even mild infections such as the common cold are associated with a marked lowering of the plasma iron level. On the other hand, the plasma iron level is of value in assessing iron adequacy; that is, if the plasma iron level is normal, manifest iron deficiency is not present and the available stores of iron are not entirely exhausted.

TABLE 3

PLASMA IRON LEVEL

	Number of cases	μg. percent
Normal	169	110 ± 31
Iron deficiency	40	26 ± 7
Infection	50	30 ± 9

Iron-binding capacity of the plasma.—As mentioned previously, the hypoferremia associated with iron deprivation is accompanied by an increase in the iron-binding capacity of the plasma (fig. 1) (*7, 15, 20, 25*). Normally about one-third of the available iron-binding protein is bound with iron. In patients with iron deficiency, the plasma iron level is decreased and the total iron-binding capacity is increased; the result is that the percent saturation of the protein with iron is reduced to less than ten. A study of the total iron-binding capacity of the plasma and the percent saturation of the protein with iron is, therefore, of value in the detection of a deficiency of iron. Although the plasma iron level is also reduced in patients with infections, the total iron-binding capacity is also reduced so that by this means anemia and hypoferremia due to iron deficiency can be distinguished from anemia and hypoferremia due to infection (*8*).

Anemia.—Manifest iron deficiency by definition is accompanied by anemia and the severity of the anemia reflects reasonably accurately the degree of depletion of iron (*34*). Since anemia results from a great variety of causes, the presence of anemia *per se* does not necessarily indicate a deficiency of iron. In iron deficiency anemia the hemoglobin is reduced out of proportion to the red cell count and volume of packed red cells. This is reflected in a reduction in the mean corpuscular volume and in the mean corpuscular hemoglobin concentration, as shown in table 4. Examination of the blood smears in such instances of microcytic, hypochromic anemia reveals small pale cells with pronounced central pallor. In extreme degrees of hypochromic anemia, most of the red cells are mere rings.

TABLE 4

IRON DEFICIENCY ANEMIA

	Volume packed red cells ml./100 ml.	Mean corpuscular volume cu.	Mean corpuscular hemoglobin concentration percent
Normal	42–52	82–92	32–34
Mild	30–40	78–82	29–32
Moderate	20–30	68–78	25–29
Severe	10–20	50–68	22–25

It is indeed fortunate that, for purposes of evaluating iron adequacy, the morphologic features described above are almost specific for iron deficiency. Of all the other forms of anemia, only thalassemia possesses similar morphologic characteristics. Thalassemia is a relatively rare condition and can be easily distinguishable from iron deficiency anemia on the basis of the physical findings, and the presence of nucleated red blood cells, stippling and numerous target cells in the peripheral blood smear. The plasma iron level in uncomplicated thalassemia is either normal or elevated, the total iron-binding capacity is reduced rather than increased and the amount of hemosiderin in the marrow is increased (*28*).

From the foregoing statements it is obvious that in any evaluation of iron deficiency it is imperative that the morphologic characteristics of the erythrocytes be determined accurately. This can be done in one of several ways. The mean corpuscular volume and mean corpuscular hemoglobin concentration can be calculated from the red cell count, the hemoglobin determination and the volume of packed red cells as outlined by Wintrobe (*34*). However, highly accurate determinations are required if the data are to be considered reliable. This fact is not generally appreciated, and few are aware that when the red cell count is performed by the common procedure of using 1 pipette and counting only in 1 chamber, the error in the determination may be as great as ±30 percent. To obtain accurate red cell counts, it is absolutely essential that accurately calibrated equipment be used, all sampling and technical errors must be reduced to a minimum, 2 pipettes and 2 chambers must be employed for the counts, between 400 and 600 cells must be counted in each chamber, and the inherent error of distribution of the cells in the chamber must be estimated. Even when all of these precautions are followed, the inherent error in the method is still ±11 percent.

Hemoglobin can be determined with an accuracy of ±2 percent by photoelectric methods providing the instrument is first accurately calibrated by the Van Slyke or some other suitable method. With proper technique, the determination of the volume of packed red cells can be made with an accuracy of ±1 percent.

From the above, it is obvious that of the three methods available for the detection of anemia the most accurate procedure available is the determination of the volume of packed red cells. This method lends itself well to large population surveys since venous blood is usually obtained for other purposes as well and many determinations may be performed simultaneously with a minimum requirement of time, skill, and experience. The enumeration of red cells is the least valuable of the three procedures because it carries the highest error, is the most time-consuming to perform, and requires the greatest skill.

From the practical standpoint, for the purposes of a large survey it is quite feasible to determine only the hemoglobin and volume of packed red cells. From these two determinations the mean corpuscular hemoglobin concentration can be calculated, thus circumventing the enumeration of erythrocytes without a serious loss in the total information gained. Indeed, it is far more valuable to have reliable information concerning the concentration of hemoglobin in the cells than to have unreliable information, gained at the expense of much more technical effort, concerning the average size of the corpuscles.

Finally, it should be mentioned that an experienced observer can detect the presence of microcytosis and hypochromia by the examination of a properly prepared blood smear. The calculated corpuscular constants should always be checked against the appearance of the erythrocytes in the stained smear.

Copper

Although copper has been known to be an essential dietary constituent for most, if not all, mammals since the mid-nineteen twenties (*3, 4*), a clearly proven deficiency state in a human subject has not yet been described. From this it cannot be concluded that such deficiency does not exist at times, because the studies have not been sufficiently comprehensive to either prove or disprove the hypothesis. The reasons for this are several. First, knowledge of the metabolism of copper has been too limited to allow clinicians to know where and how to intelligently seek out such a deficiency. To clearly demonstrate that depletion of copper exists, as will be discussed later, it is essential to show that the plasma copper level is markedly reduced. Few investigators have performed such determinations. Finally, most workers have concerned themselves with a comparison of the rapidity of blood regeneration in subjects with microcytic, hypochromic anemia following the administration of iron alone, and of combined iron and copper therapy. A voluminous but inconclusive literature has been accumulated on this subject. When consideration is given to the many difficulties encountered in properly controlling and evaluating such a study, it is not surprising that the results have been equivocal. Nor does it seem likely that in the future this same type of study will be productive. Anemia as a consequence of copper inadequacy would represent a high order of copper depletion. What is needed are techniques for detecting partial depletion of copper reserves.

In balance studies it has been demonstrated in the normal adult that positive copper balance is maintained if the dietary intake is approximately 2 mg. per day or greater (*4*). The dietary requirement for children is about 80 μg. per kg. of body weight per day. An American diet of even mediocre quality contains from 2.5 to 5.0 mg. of copper per day. Because copper is widely distributed in foodstuffs and is readily stored in the body, in order for an adult to become depleted in copper it would be necessary to consume a diet so low in calories that death due to caloric inadequacy would supervene long before a serious depletion of copper could develop. Although both human and bovine milk are low in copper, and although it might be anticipated that the copper requirement of the rapidly growing infant would be high, this eventuality seems to have been anticipated by the normally high fetal stores of copper. These are 5 to 10 times higher than in the adult and are apparently sufficient to compensate for the reduced intake during the first few months of life. It would follow from all of this that a deficiency of copper, if it exists at all, must be extremely rare.

Since copper deficiency has not yet been described in human subjects, it would seem to be profitable to summarize the manifestations of copper deficiency in animals (*14, 19*). With an understanding of

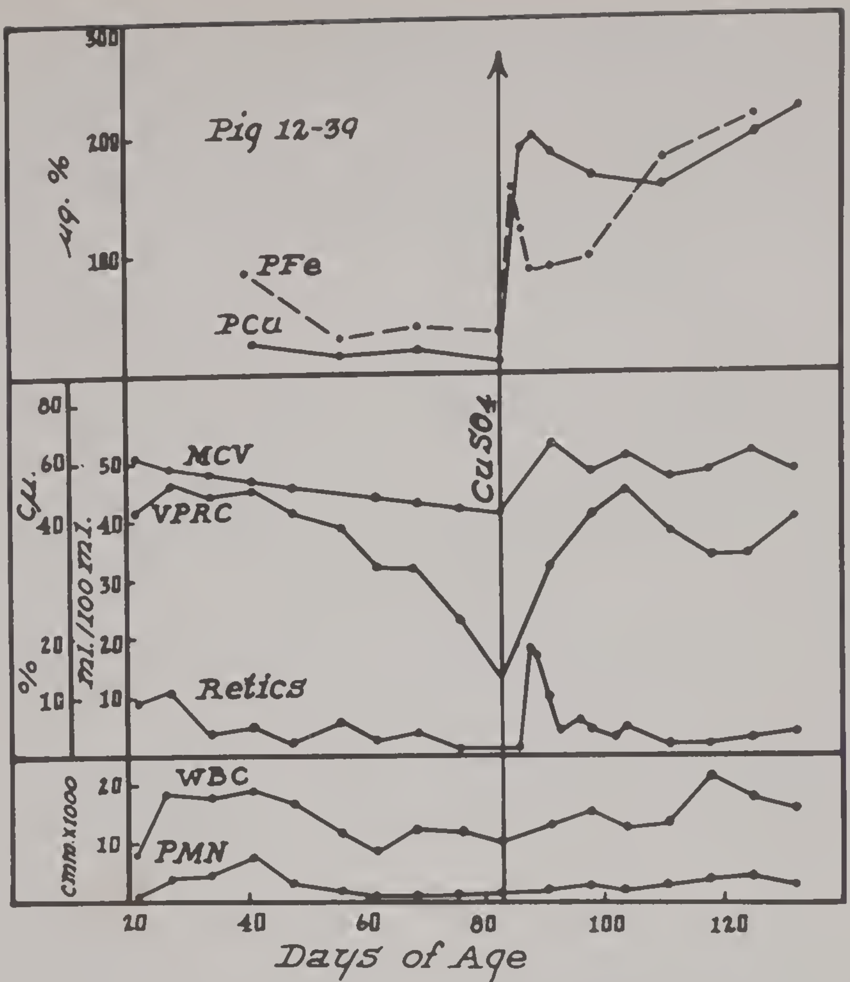

FIGURE 2.—Development of hypoferremia, hypocupremia, microcytic hypochromic anemia, leukopenia, and neutropenia in a copper-deficient pig and the response to the oral administration of 0.5 mg. of copper/kg. of body weight/day.

these, one can more intelligently know what to look for in human subjects.

When swine, 8 to 10 days of age, are fed a milk diet supplemented with adequate amounts of iron, the first manifestation is a marked reduction in the plasma copper level. Within 2 weeks after the beginning of the experiment, the plasma copper level decreases from about 185 μg./100 ml. to less than 25 μg./100 ml. Thereafter, the levels fluctuate between 0 and 25 μg./100 ml. This is illustrated in a single animal in figure 2. Likewise, in rats the plasma copper is extremely sensitive to a decrease in copper intake (*13*). As illustrated in figure 3, when adult male rats are fed a diet low in copper the plasma copper levels begin to decline immediately and reach a minimum value in 20 to 25 days. From this it may be concluded that the plasma copper level is a sensitive indicator of copper depletion.

The reduction in the plasma iron level in copper-deficient swine (figure 2) occurs in spite of the fact that the copper-deficient animals receive 30 mg. of iron/kg. of body weight/day from the beginning of the experiment. The level of the iron in the plasma is reduced to levels comparable to those observed in iron-deficient swine (table 5). As in iron deficiency, there is an increase in the total iron-binding capacity of the plasma.

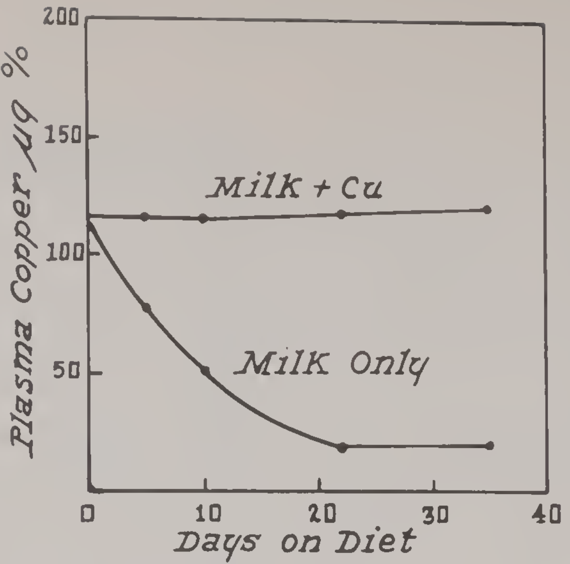

FIGURE 3.—Rapid development of hypocupremia in adult male rats fed a milk diet. (*Am. J. Physiol.*, *171*, 652 (1952)).

TABLE 5

COPPER DEFICIENCY IN SWINE COMPARISON WITH IRON DEFICIENCY

Determination	Control	Copper deficient	Iron deficient
Volume packed red cells ml./100 ml	42	22	21
Mean corpuscular volume cu μ	56	39	36
Mean corpuscular hemoglobin concentration, percent	33	29	28
Red blood cell copper μg./100 ml	110	67	110
Mean corpuscular copper μ μ μg	61	26	39
Plasma copper μg./100 ml	186	15	207
Plasma iron μg./100 ml	175	38	30
Total iron-binding capacity μg./100 ml	511	628	864

Anemia invariably develops in all swine deficient in copper. During the first month, there is little or no decline in the hemoglobin or volume of packed red cells (V. P. R. C.). Thereafter, there is a precipitous fall in these values, and, if the animals are not treated, the hemoglobin decreases from 15 to 2 gm./100 ml. and the V. P. R. C. from 40 to 8 ml./100 ml. in about 40 days. The animals become extremely pale and weak, the respiratory rate increases, and death supervenes, apparently as a result of tissue anoxia. The type of anemia which develops is microcytic and hypochromic, as indicated by a substantial decrease in the mean corpuscular volume (M. C. V.) and in the mean corpuscular hemoglobin concentration (M. C. H. C.). Thus, the anemia is of the same type as that which develops as a result of iron deprivation.

The amount of copper in the red cells in the severely deficient animals is reduced. However, this reduction takes place late in the course of the deficiency, some time after anemia becomes manifest and the reduction is not as great in degree as that which occurs in the plasma copper level. At the height of the deficiency the mean value is about 40 percent lower than the value obtained in the control group. It must be concluded from this that the erythrocyte copper is a much less labile fraction than the plasma copper and that a slight or moderate reduction in the erythrocyte copper level represents a severe degree of depletion.

The manifestations of copper depletion in swine are summarized in table 5.

On the assumption that the manifestations of copper depletion in human subjects would be similar to those in animals, the following determinations would be of value in assessing the copper status of human subjects: (*a*) plasma copper, (*b*) plasma iron, (*c*) total iron-binding capacity of the plasma, (*d*) examination of the blood for microcytic, hypochromic anemia, and (*e*) erythrocyte copper. In order to conclusively demonstrate a severe deficiency of copper, one should add to these criteria a poor or negligible response of the anemia to the administration of iron (spectroscopically free of copper) and a prompt response, with an accompanying reticulocytosis, to the administration of copper and iron.

Over the past 10 years we have measured the plasma copper level in 228 normal individuals (table 6) and in approximately 300 patients with a variety of disorders (*17*, *18*). Hypocupremia has been observed consistently in only three situations; namely, the newborn (*12*) the nephrotic syndrome (*6*), and in patients with hepato-lenticular degeneration (table 7) (*2*, *5*). In addition, hypocupremia has been

TABLE 6

Normal Plasma Copper

μg./100 ml.	Number of patients	Percent
90+	218	96
80–90	8	3
68–79	2	1

TABLE 7

Hypocupremia in Human Subjects

	Number of cases	Plasma copper mean μg./100 ml.	Plasma copper range μg./100 ml.
Normal	228	116	68–161
Newborn	14	75	45–110
Nephrotic syndrome	16	64	20–96
Hepato-lenticular degeneration	8	51	33–71
Sprue	1	16	
Idiopathic hypoproteinemia	1	60	

observed in 1 patient with severe sprue (*5*) and in 1 patient with a rare and poorly understood disorder, idiopathic hypoproteinemia (table 7). In the newborn and in patients with hepato-lenticular degeneration it has been adequately demonstrated that the hypocupremia is not associated with a depletion of tissue copper. Indeed, in both conditions tissue copper is markedly increased (*4, 5*).

Studies on patients receiving diets inadequate in at least certain respects, such as patients with scurvy, cirrhosis of the liver, and repatriated American prisoners of World War II, have revealed either a normal or an increased level of copper in the plasma (table 8). Likewise, as shown in table 8, the plasma copper level has not been found to be reduced in either infants or adults with a microcytic, hypochromic anemia as a consequence of iron deficiency. Thus, no evidence has been obtained for the existence of even a mild state of copper depletion in human subjects as the consequence of an inadequate dietary intake of copper. Indeed, we have fed diets identical to those which were successful in producing copper deficiency in rapidly growing swine to 2 infants, 5 days and 8 months of age, respectively (table 8). Although this experiment was continued for 4 and 5 months, respectively, the plasma copper level remained normal and no anemia developed. This may have been due to the fact that the growth rate of infants is less rapid, or that their copper stores are greater than in young swine; or perhaps the human requirement for copper is less than that of swine. In any event, our experience suggests that copper deficiency as the consequence of an inadequate dietary intake of this element must be very rare in man.

TABLE 8

THE PLASMA COPPER LEVEL IN PATIENTS WITH VARIOUS FORMS OF NUTRITIONAL DEFICIENCY

Condition	Number of patients	Mean plasma cu. µg./100 ml.	Range plasma cu. µg./100 ml.
Normal	228	116	68–161
Cirrhosis	7	136	101–159
Scurvy	2	169	155–183
Malnutrition [1]	38	148	92–215
Iron deficiency adults	9	132	102–164
Iron deficiency infants	26	168	117–225
Milk diet [2]	2	[3] 143	109–178
Nutritional hypoalbuminemia	1	155	----------

[1] Repatriated American prisoners of World War II.

[2] Fed a specially prepared milk diet low in copper for 4 and 5 months, respectively.

[3] At the end of the experimental period.

Although dietary deficiencies of copper may not occur in man, there could be certain conditions (conditioned deficiencies) in human subjects in which copper might not be absorbed or might be excreted in excessively large quantities from the body. With this possibility in mind, we have determined the plasma copper level in several patients with sprue. One of these patients (*5*) who had a severe microcytic, hypochromic anemia was found to have a plasma copper level of 16 μg./100 ml., a red cell copper level of 83 μg./100 ml. of packed cells, a plasma iron level of 12 μg./100 ml., and a total iron-binding capacity of 360 μg./100 ml. Unfortunately, during the baseline period the patient improved markedly on dieto-therapy and hospitalization and the copper levels had already begun to increase prior to the administration of copper. Although it seems likely that this patient was depleted of copper as well as iron, this could not be proven because it was not demonstrated that the anemia would not respond to iron alone and required iron plus copper therapy. This observation does, however, indicate an area where further investigations may be worthwhile.

Since approximately 96 percent of the plasma copper in a normal adult subject is in the form of a blue α globulin, ceruloplasmin, it would seem not unlikely that this copper protein might be excreted in large quantities in the urine of patients with proteinuria. If the loss were great and extended over a prolonged period, it is conceivable that depletion of the body stores might occur. Therefore, to investigate whether a conditioned deficiency of copper takes place in patients with massive proteinuria, plasma copper, erythrocyte copper, the urine copper, and the hemitologic status of a group of patients with the nephrotic syndrome were studied (*6*). The results are summarized briefly in table 9.

The loss of copper in the urine was considerable as compared with the normal and amounted to 0.1 to 0.5 mg./day. Hypocupremia was present in 4 of the 6 patients. In 3 of the 5 patients in whom the amount of copper in the erythrocytes was determined, the value was found to be reduced. In one the extremely low value of 40 μg. was obtained. Anemia when present was normocytic and normochromic rather than microcytic, hypochromic. Three patients were treated with copper sulfate orally and in none was a demopoietic response observed. Thus, from the limited data available it seems unlikely that the anemia in these patients was related to a deficiency of copper.

Additional studies are needed before the degree of copper depletion in patients with the nephrotic syndrome can be evaluated. The hypocupremia may be due solely to an inability to synthesize ceruloplasmin in the necessary amounts and the copper stores may not be depleted to any significant degree. On the other hand, the reduction in erythrocyte copper in several of the patients suggests that in at least certain of the tissues of some patients there may be a significant depletion of copper. To answer this question, it will be necessary to determine the tissue stores of this element directly.

TABLE 9

STUDIES ON COPPER METABOLISM IN PATIENTS WITH THE NEPHROTIC SYNDROME

Patient	Urine cu. µg./day	Urine protein gm./day	Plasma cu. µg./100 ml.	Red blood cells cu. µg./100 ml.	Volume packed red cells ml./100 ml.	Mean corpuscular volume cu.	Mean corpuscular hemoglobin concentration percent
Normal	9	0. 1	116	115	49	87	34
C. E	270	8. 8	63	94	33	82	35
R. K	490	15. 4	58	61	42	88	32
G. H	346	12. 2	94	----------	30	80	35
W. B	183	5. 6	89	87	30	88	35
D. G	324	18. 4	49	71	43	89	34
E. C	92	9. 3	20	40	26	88	35

Cobalt

Normal adult subjects on self-selected diets consume about 5 μg. to 8 μg. of cobalt per day (*16*). Seventy-three to 97 percent of this amount is absorbed and about 67 percent of the total daily intake is excreted in the urine. Positive balance is maintained on as little as 5 μg. per day. In view of this extremely low daily requirement, it is not surprising that a deficiency of cobalt *per se* has never been recognized in a human subject.

Little is known of the functions of cobalt in the body. It has been demonstrated to be essential for the activity of certain enzymes (*29*) and the discovery (*27*) that it is an integral part of the vitamin B_{12} molecule is of considerable interest to hematologists. The experiments in the field of animal nutrition mentioned below add still further interest to the role of cobalt in erythropoiesis.

Cobalt deficiency in ruminants grazing on pastures of low-cobalt content has been recognized for some time (*3*). The deficiency syndrome is characterized by unthriftiness, anorexia, severe anemia, and finally death. The curious observation (*21*) that cobalt was effective when administered orally but not parenterally was not understood until recently when it was demonstrated by several groups of workers, (*1, 9, 22, 23, 30*) that the condition is rapidly and completely alleviated by the parenteral administration of about 50 μg. of vitamin B_{12} per day, an amount, incidentally, which is much greater than that required for the treatment of pernicious anemia. Thus the deficiency syndrome in sheep grazing on cobalt-deficient pastures is, in effect, the result of vitamin B_{12} deficiency.

Pernicious anemia and the related macrocytic anemias which respond to the parenteral administration of small amounts of B_{12} are due, in the sense that a specific cobalt-containing dietary compound is lacking, to a deficiency of cobalt. Macrocytic anemia and megaloblastic bone marrow are the distinguishing features of this group of diseases. In addition, the serum and urine vitamin B_{12} levels are markedly reduced in untreated patients (*24*). Microbiological assay for vitamin B_{12} in serum and urine, particularly by the method which utilizes *Euglena gracilis* as the test organism, offers a delicate and apparently reliable method for the evaluation of vitamin B_{12} adequacy, and in an indirect way for a specific type of "cobalt adequacy."

Zinc

Normal values for zinc in blood have been published by Vallee and Altschule (*32*) and Vikbladh (*33*). Detection of a zinc deficiency should be possible by a study of the concentration of this element in plasma, erythrocytes, and leukocytes. The only studies of this nature in patients with nutritional deficiencies have been performed by

Eggleton (*10, 11*). He found subnormal amounts of zinc in the blood of Chinese subjects afflicted with beriberi, nutritional edema, and semi-starvation. The zinc content of the toenails, fingernails, and skin was reduced to about half the normal value. However, Vikbladh more recently has shown that the plasma zinc level is also moderately depressed in patients with infections. Since it is entirely likely that some of Eggleton's subjects had infections, some doubt may be cast on the interpretation of his data. Indeed, Eggleton noted the content of zinc in the livers and kidneys to be increased rather than decreased.

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Other Functions

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In a consideration of the effects of various trace elements upon animal metabolism other than hematological effects, particularly pathological changes, I shall limit my discussion today to copper, molybdenum, manganese, and cobalt. Since all of the elements have interactions with phosphorus, mention of phosphorus will of necessity come into the picture.

While all of these elements have recognized hematological effects, other changes which develop and which do not appear to be the results of an anemia are sometimes quite striking. My interest and the interest of those working with me in the Nutrition Laboratory at the University of Florida have been concentrated on changes which occur in pigmentation, bone and teeth, heart, and reproductive effects. This discussion today will be largely limited to discussion of these changes which occur with a disturbance of the trace element, nutrition.

One of the most curious effects which develops with a deficiency of copper, particularly as it is associated with the presence of a small amount of molybdenum in the diet, is the loss of normal pigmentation. This is true in cattle, sheep, rats, rabbits, possibly in guinea pigs, and there is at least one report on dogs (*2*, *5*). With a deficiency, there develops a gradual loss of pigmentation of the melanin type, and the animal undergoes a "fading" or a true achromotrichia. Under practical feeding conditions in many parts of the world, cattle and sheep grazing on pastures which are now known to be low in copper develop this fading or "lime hidedness" or achromotrichia with the result that black animals such as the Angus assume a gray color, red animals such as the Hereford become a sort of tan, and many in-between colors develop even to the famous lavender or purple cow which was actually a black cow that assumed a lavender tinge.

While it is true that anemia usually precedes or accompanies this change in the pigmentation of hair coat, there is reason to believe that the anemia is not the cause of the loss of hair coat color. Much of this work develops out of studies made with rats, in which it was possible to cause the loss in hair coat color but not developing a severe anemia, and in which it was possible to demonstrate a relationship between copper and pantothenic acid (*8*).

In studies with rats on comparatively low copper intakes with molybdenum included in the ration, rats were carried to maturity with a rapid fading of the hair coat color and when the animals were showing severe achromotrichia, pantothenic acid was administered to some and copper to others. It was possible to show that pantothenic acid had a marked influence on one part of the animal body, whereas copper had a marked influence on the entire body.

This interrelationship points to a cooperative effect of copper and pantothenic acid in the development of pigment (*3*), and while this is thoroughly discussed in the Symposium on Copper Metabolism (*2*), it must be admitted that the exact details of this interrelationship are yet to be explained. The natural reaction of humans is: "Does copper have any relationship to the natural development of gray hair in older people?" And to this the answer apparently must be negative. At least so far as I know, no one has been willing to undergo a severe copper deficiency regime in order to discover whether his hair will turn gray, and conversely can be cured by addition of copper to the diet. The other test, that of consuming copper as a means of increasing pigmentation in the hair has been tried, with so far as I know, no positive results.

That the relationship of copper to pigmentation is not the only effect in the keratin tissues of the body is pointed up by the work of Marston and his coworkers (*5*) in Australia. They have pointed out that copper is primarily responsible for the catalytic oxidation of the –SH group in prekeratin to the –SS group of keratin in wool. It is probable that a failure of this particular reaction may explain the so-called loss of crimp or development of "steely" wool in sheep.

This is a very interesting condition, particularly in the Merino areas of Australia, and has made it possible for the Australian workers to locate copper-deficient areas by examining the wool in the auction sales and upon discovering a wool which has lost its crimp, tracing it back to the copper-deficient source. Here again, there seems to be no direct relationship to humans, where its logical parallel would be the loss of curl in hair if subjected to a copper deficiency condition.

While pigmentation changes are a very useful tool in the detection of copper deficiency in livestock, they do not in themselves constitute a particular hazard to animals. However, with the development of achromotrichia there are also other changes in animals which are more severe and which have at least counterparts in humans if not direct parallels. Primary among these changes are those which occur in bones.

My introduction to this phenomenon occurred in 1943 when I was visiting on a farm in south Florida and was shown a newborn calf with what appeared to be a full blown case of rickets. There were swellings at all of the joints, and the rachitic rosary was very pronounced. There was a marked failure of calcification.

The owner informed us that he had a large percentage of calves born this way and had been unable to overcome the difficulty by feeding large amounts of calcium and phosphorus in the form of bone meal. Later, in experimental work at the Everglades Experiment Station, we were able to demonstrate that on a low copper ration, containing about 3 p. p. m. of copper and between 2 and 3 p. p. m. of molybdenum with the phosphorus level at an adequate 0.2 percent of the dry matter in the feed, almost 10 percent of the calves were born with skeletal deformities.

Further, we were able to show that in 100 percent of the calves, nursing cows on pastures which were deficient in copper, there developed a swelling of the ends of the long bones, grossly indistinguishable from rickets or a scorbutic-like syndrome. Our first move was to analyze the blood of these animals for vitamin C. We were unable to demonstrate any deficiency of this vitamin and necessarily were hard put to explain rickets with the calves receiving whole milk and certainly adequate sunshine. In the course of our investigation we discovered that with inclusion of a small amount of copper, in the case of the calves, approximately 0.3 of a gram of copper sulfate per day, the rachitic symptoms disappeared rapidly and no new symptoms developed.

These observations have led to a large number of studies on the effects of copper and molybdenum and their interrelationships with phosphorus and possibly with cobalt (*2, 6, 7*). In older animals grazing on similar pastures, there is a rapid depletion of the phosphorus and calcium from the bones with the result that these animals are subject to multiple fractures and it is not uncommon to have cattle exhibit from 1 to 20 broken bones when brought to slaughter or autopsy.

In an effort to ascertain the effect of copper and molybdenum on phosphorus in the animal body, we have carried out balance studies on cattle, rats, and rabbits, in which we have used labeled phosphorus, copper, and molybdenum, in different approaches, in an effort to ascertain the effect of these elements on one another. In rats we have not been able to detect an effect of molybdenum or copper upon phosphorus metabolism that would explain the rarefication of bones which occurs in rabbits and in cattle. In rats that have not yet calcified their bones, we have been able to observe that the presence of added molybdenum with insufficient copper will result in a failure of calcification.

In cattle the presence of added molybdenum with insufficient copper will result in a rapid loss of phosphorus from the bones, and concurrently a marked increase in the alkaline blood phosphatase with the result that the animal is thrown into negative phosphorus balance and will eventually develop bones that contain between two-thirds and three-fourths of normal mineral content. The breaking strength may drop from a normal 3,000 to 4,000 pounds to approximately 300 pounds under the conditions of our testing.

In an effort to explain the interaction of these trace elements, we have aproached the study from a number of angles. At first we assumed that perhaps the molybdenum was combining with the phosphorus in the intestinal tract and preventing its absorption from the tract with the result that the animal was deprived of normal phosphorus intake. Through the use of phosphorus 32 and molybdenum 99 we were able to show that this was not the case and that regardless of the level of the two elements in the diet, absorption of phosphorus was essentially the same under conditions of zero and very high molybdenum intake. The next assumption which we followed up was the possibility that molybdenum was combining with phosphorus in the body and preventing its deposition in the bones (*4*, *6*). This could not be demonstrated.

We did discover that when low copper intakes were accompanied by feeding of molybdenum, there was a rapid deposition of molybdenum in the bones, presumably in place of or as a part of the phosphorus deposition process. However, the molybdenum did not remain in the bones with phosphorus but was rapidly remobilized and carried out of the body. When we incorporated adequate copper, molybdenum was eliminated rapidly but was not first deposited in the bone.

This apparent competition or replacement of phosphorus with molybdenum in the bone but with the failure to form a stable bone salt may be the explanation for the loss of osseous tissue under conditions of copper deficiency with presence of molybdenum excess. We have not been able to demonstrate the same rapid loss of bone tissue with a simple copper deficiency but without the presence of molybdenum in the diet.

Because we observed a severe arthritis developing in older animals when they were on diets which were low in copper and which contained small amounts of molybdenum, we have undertaken additional studies with laboratory animals as a means of determining wherein this relationship occurred. Using rats as experimental animals, it was possible to show that the severe molybdenosis or copper deficiency syndrome could be corrected by including vitamin B_{12} in the ration or by removal of the adrenals (*6*). This picture has been further complicated by the fact that female rats have been much more susceptible than male rats to our experimental diets, indicating that the sex hormones may also play a role in the functioning of these trace elements in the utilization of the major elements, particularly phosphorus.

Because we were not able to produce the same changes in rats that we observed in cattle, we shifted our experimental program to the guinea pig and rabbit with particular emphasis on the rabbit, and we have been able to produce bone changes which are suggestive at least of those which have occurred in cattle (*1*).

Under conditions which are reasonably similar, we have observed that the bone calcification is disturbed and that we produce abnormally formed bones and that apparently this change is due to the molybdenum being deposited and then removed because of the failure to form a stable osseous tissue.

The fact that vitamin B_{12} served to alleviate symptoms of molybdenosis or copper deficiency in rats led us to investigate the value of cobalt in the prevention of the syndrome in cattle. Earlier, we had conducted studies in which cobalt was used without appreciable effect. A series of experiments were undertaken in which cobalt was administered orally and the vitamin B_{12} was injected intravenously in order to discover if we might cause a change in the copper and molybdenum metabolism of cattle. These results indicated that including cobalt in the diet greatly increased the efficiency with which copper was utilized by cattle. but that it did not significantly change the relationship with regards to molybdenum, other than to reduce the level of copper necessary to protect the animals from a molybdenosis.

These studies brought to light a further condition which. while it still baffles us, is serious insofar as individual cattle are concerned and which appears to be related to a complex interrelationship of copper, molybdenum, cobalt, and perhaps manganese, phosphorus, and calcium. In a number of circumstances we have observed that the teeth of young and old cattle rapidly deteriorate while placed on pastures known to be copper deficient, but also suspected of being rich in calcium and borderline in phosphorus. The typical picture seen is one of fluorosis except that the incisors are not affected, but the molars are. In these cattle, within a year's time, the teeth may be eroded to the gums.

We have eliminated the possibility of fluorosis by extensive analyses of the bones, teeth, and feed, and have had to assume that this condition is due to an entirely different set of circumstances. Since the workers in Australia have demonstrated that a ratio of calcium to phosphorus of approximately 1 to 3 to 1 to 5 will result in a syndrome in sheep that resembles fluorosis, we investigated the ratio of these two elements under these conditions. We discovered that rather than a ratio of low calcium-high phosphorus, we were dealing with a high calcium-low phosphorus situation accentuated by the presence of small amounts of molybdenum, small amounts of copper, and perhaps a deficiency of manganese and cobalt.

At the present time we have not been able to completely solve this problem. although we do appear to have alleviated the situation by insisting on high phosphorus feed and supplements including liberal levels of cobalt and manganese in the diet. Our best lead so far has been with regards to habits of ruminants in which they regurgitate rumen contents. We have observed that with a cobalt deficiency, we may have a change in the pH of the rumen. While we have not yet had sufficient evidence to confirm our theory, we suspect that under these circumstances we have a lowering of the pH, and when the animal chews its cud it is essentially chewing an acid media which may result in the erosion of the molars. This is at least an intriguing theory and we hope within the course of the next 2 years to obtain data that will either confirm our theory or further confuse the problem.

Because bone changes may be caused by many different factors, it is difficult to say that these conditions observed in animals have or have not any relationship to similar conditions seen in humans. They are presented here as examples of changes which may occur because of a slight imbalance of trace elements which are normally consumed in very small amounts.

Two other phases of this work with trace elements would seem to have the bearing on the possible relationships with humans. One of the conditions observed in our animals on experimental pastures, low in copper with small amounts of molybdenum, is an occasional case of "falling disease." This is a cardiac failure associated with massive degeneration of the muscle fibers of the ventricle. This myocardial degeneration was first suspected when it was observed that all of the cattle dying in this way had extremely flabby heart tissues.

My observance of this condition occurred first when riding a range with a rancher in south Florida. He pointed out to me a three-year-old heifer which he said was "fat as she could roll", and which he was driving up to the corral preparatory to sending her to market. As we rode along, the cow was observed to stumble and fall almost as if struck by a hammer. Before we reached the cow she was dead. The rancher quickly drew out his knife, slit her throat. took his horse, threw a rope around her legs, dragged her over to the nearest tree and hauled her up for butchering.

I was especially intrigued to observe the internal organs. The only observation of gross pathology was the slightly enlarged, very flabby heart which had on its surface a few small petechiae. I was informed that this condition occurred frequently in their cattle when they were being rounded up to send to market and that the hands had no objection whatever to eating the meat. They had found by experience that it was perfectly all right with no bad after effects.

Subsequently, we were able to produce this condition experimentally by depriving animals of copper until they were in a severe case of copper deficiency, administering copper as a therapeutic treatment, subsequently removing the copper before a store of this element could be built up and keeping the animals on a deficient pasture with a minimum intake of phosphorus.

In our subseqennt work, we have come to the conclusion that it is necessary to have both a deficiency of phosphorus and copper to produce this heart failure in cattle. The explanation of this phenomenon, however, is much more elusive, and at the present time we are actively engaged in studying the changes which occur in the enzymes of the heart tissue under conditions of different levels of copper intake. It appears that some severe change does occur inasmuch as the muscle fibers show a degeneration similar to that which is observed with vitamin E deficiency in other species.

You will remember that in beginning this discussion, I mentioned that I had been initiated into the study with the observation of a newborn calf with a full blown case of rickets. Subsequently, we observed many monstrosities produced by cattle on diets low in copper with the presence of a small amount of molybdenum, low in phosphorus but still within the normal range and borderline or low (5 to 15 p. p. m.) in manganese.

We observed that in many of these animals bones were completely missing, particularly the femur. Occasionally, the head bones were not calcified. This development of abnormal young has led us to a considerable study of placental transfer that may be influenced by copper and molybdenum, and, in this respect, manganese has also come into the picture. We have observed in studies with rats, pigs, and cattle that calcium readily crosses a placental barrier whereas molybdenum is almost completely barred.

With the presence of molybdenum in the diets, phosphorus is severely impeded in crossing the placenta and copper is likewise interfered with. Thus, we appear to have a situation in which with a small amount of molybdenum (levels of 3 to 5 p. p. m. in cattle and 20 to 40 p. p. m. in rats, and 80 to 140 p. p. m. in swine, and higher levels in rabbits), calcium readily crosses the placental wall, but molybdenum does not cross, and by its presence prevents adequate phosphorus and copper and manganese from crossing to the fetus with the result that we have abnormal bone development.

This work has not yet proceeded to the point where we can produce monstrosities at will, although in our work with swine, we have been able to produce abnormal young in at least a portion of many litters.

The presence of molybdenum appears to prevent the utilization of manganese in the diet, with the resulting development of abnormal bone calcification.

This observation developed in studies with rabbits in which it was observed that the bone changes which developed with feeding of molybdenum resembled very closely those reported for the changes in rabbits on manganese-deficient diets. Consequently, we endeavored to prevent the development of bone deformities by including extra high levels of manganese in the ration, and, at least in some measure, we were successful in counteracting the effects of molybdenum.

In the development of the fetus, we were unable to obtain comparable manganese transfer across the placenta when larger than normal amounts of molybdenum were present in the diet.

Certainly these results with animals cannot be translated directly to humans; however, they do point out certain fundamental interrelationships in the metabolism between the trace elements and phosphorus, more than with calcium, that must be considered as potential changes which can occur in many different species, and possibly also in the human species. Fortunately, for the human, the presence of adequate copper is a usual circumstance and presence of abnormal amounts of molybdenum is not too common. The fact that adequate copper is necessary for the proper utilization of phosphorus does point out that changes which prevent the adequate utilization of trace elements may very well result in the improper metabolism of phosphorus and calcium.

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Discussion

COMMANDER R. A. PHILLIPS (Bureau of Medicine and Surgery, Department of the Navy):

I would like to ask Dr. Cartwright if there are any observations on the plasma iron and the plasma copper level in the newborn and patients with nephrosis who have an infection.

CARTWRIGHT:

First of all, we have no observations of infections in the newborn. We have observations of nephrosis patients with infection since they are so prone to it. The influence of infection is very difficult to detect since these patients also lose large quantities of iron-building protein in the urine—even larger quantities than the copper protein—so most of them have hypoferremia even without infection, so you do not expect the infection to influence the level.

Apparently, in the patients we have studied with nephrosis—approximately 20—we have not been able to observe any influence of the present infection on the copper level. I would take that to mean that other forces were greater than the forces of infection, which is unable to cause a rise.

A. A. ALBANESE (St. Lukes Convalescent Hospital):

Are the hemoglobin values of nephritics generally lower than normal?

CARTWRIGHT:

Yes, they are. Unfortunately, I did not have time to present those data. Most of our patients with nephrosis have had a mild degree of anemia. That anemia has also been normocytic and normochromic, not microcytic and hypochromic, and we have not observed a response to the administration of copper insofar as the anemic condition is concerned. I would conclude from that that the anemia was present but was not related to copper depletion.

QUESTION:

Are there other tissues besides the cardiac muscle that degenerate in laboratory animals?

DAVIS:

We have not been able to find them. We have searched pretty thoroughly some hundred tissues but so far have not been able to find degeneration in any other than the cardiac muscle in these particular animals

HORWITT:

I would like to ask, was the alpha tocopherol of these animals known?

DAVIS:

At the suggestion of Dr. King in the early part of this work, we ran down the vitamin C story very well. We found they were perfectly normal as far as ascorbic acid was concerned. There are many relationships in the trace element. I didn't begin to discuss the traces of manganese and the circumstance in which we occasionally find extremely high copper levels in the liver of the animal while at the same time the animal is exhibiting a copper deficiency syndrome. Apparently, there are conditions where the copper present is not rendered useful to the animal and manganese apparently also fits into that picture.

THURSDAY DINNER SESSION

25 February 1954

Lieutenant Colonel C. J. KOEHN (Quartermaster Food and Container Institute):

The subject of our speaker tonight is a nutrition survey which he and I made in Korea last spring and summer. He will give you the scientific details of our findings and I will give you the background of this survey.

We were alerted by the Surgeon General that we would be sent to Korea, and were called in for a briefing. We wondered what we had done to deserve such an assignment. We were informed that the Far East Command had requested that two nutritional consultants be sent to Korea to make a nutrition survey of the Republic of Korea Army, of the United Nations' prisoners of war, and the civilians of South Korea.

That sounded like a tremendous job and it sort of floored us. We wondered whether two men were supposed to accomplish this in the period of 100 to 120 days allotted. They gave us aid and comfort, but no information as to what help we would get on the other side.

Dr. Sandstead and I went back to our respective bailiwicks and corresponded, wondering how much we could accomplish. We didn't have much time to worry, though, because we received teletype orders to report to the west coast within 2 days; so I borrowed a pair of skin calipers from Lieutenant Colonel Ryer and Dr. Sandstead got his clinical gear together and we took off.

When we arrived at the Far East—that is, Japan—we were briefed by the Chief Surgeon, who told us that they really expected us to do a complete job; in other words, everything that was necessary for a complete nutrition survey. We were called before General Mark Clark who further briefed us and told us he wanted the facts and figures as to what was happening to the various population groups, what their nutritional status was, their nutritional requirements, what the adequacy of the ration was, and to come up with concrete recommendations for any corrective action we thought necessary.

He also told us that we were to have all material aid and logistic support which we needed, and said it would be forthcoming from the Eighth Army in Korea.

We set our plans to do a full-scale survey which involved laboratory procedures, clinical evaluation, dietary analysis, anthropometric measurements, and anything else we could think of at the time. We had to wait for clearance to Korea, which gave us 7 days to gather information from the Japanese who had occupied Korea for a number of years and should have had quite a few scientists who had know-how in that particular field. However, they had very little information and no anthropometric data that was of use to us.

We contacted the 406 Medical Laboratory in Japan, which furnished support to the field laboratories in Korea, and they promised us a mobile field laboratory truck which was then in Korea. They promised that if we sent samples of blood serum to them, they would run vitamin A, carotene, and ascorbic acid analyses and furnish us with shipping cases. They promised a steady supply of dry ice and courier service from the First Medical Laboratory near Seoul to Tokyo.

We proceeded to Korea and were assigned to the First Medical Field Laboratory just outside of Seoul at Yong Dong Po for logistical support. There we started to gather our equipment, which consisted of one two-and-a-half-ton laboratory truck which we had to completely strip down and re-equip, one 6 x 6 two-and-a-half-ton truck which carried tentage, bedding, rations, sleeping bags and all the gear that was necessary for a self-sustaining outfit in the field and two jeeps. We gathered together, borrowed and stole from various units, our medical team, of which Dr. Sandstead was the Medical Officer in charge, and I the Executive Officer. We had one captain for administration and supply officer, one medical corps officer as assistant surgeon, two Republic of Korea majors, Medical Corps, who served as interpreters and clinical assistants, two enlisted laboratory assistants, two drivers, and one clerk.

We discovered the trouble we were going to run into very soon—in fact the first Sunday we were in Korea while awaiting the arrival of the two ROK majors who were to act as interpreters. We waited for quite a while and then I told Dr. Sandstead I had to go to the laboratory and check the equipping of the field truck. He excused me and, as I was leaving, I saw two ROK majors approaching and I asked them if they were to work with the American team.

They said, "Colonel Sandstead?" I said, "Come with me." I introduced them to Dr. Sandstead and as I stood around a while, it became apparent that the interview was going very badly. Everybody was talking on a different subject and it became a little amusing, almost ludicrous. I was afraid I would spoil it all by laughing, so I went on about my business.

In about 3 hours I came back and I could hear voices in the distance; Dr. Sandstead was arguing with one of the majors very vehemently. I heard him saying, "You're in the Army now and you are going with us on this tour."

Then the major said, "No, I stay in Seoul."

These two majors were to have been sent from Taegu, but one of them kept talking about Seoul all the time, so I asked him, "Are you from Taegu?"

He replied, "I am stationed in Seoul and I have got to go back to Seoul."

I said, "You are not an interpreter?"

"No, I am just bringing this man down to introduce him to Colonel Sandstead."

Well, we dismissed them and laughed about it. But, it was an indication that there were more serious difficulties ahead, and we decided that all we needed was two more interpreters to interpret for the interpreters.

We organized the team within 1 week, which I think we can be proud of. We could have done it even sooner, except for a series of misfortunes which befell the truck, including catching on fire and the tires falling off, but we were on the road a week after we hit Korea.

Our first voyage was directly north from Seoul up to the western line, for a twofold purpose. One was to shake down our equipment to see whether or not it would stand the roads, whether or not we had the proper chemicals and equipment along, whether our personnel were properly trained in the laboratory procedures, and also to study the Korean Service Corps, which is attached to the American divisions, to try to establish a base line for our study of the ROK Army and prisoners of war.

We had no mishaps, fortunately, and returned to Seoul to refurbish our equipment and adjust any deficiencies which we noted in our equipment. We knew we had a great deal of ground to cover in the next 2 months, most of Korea, and the local weather prophets had forecast that the rains were going to come any day. Since our laboratory truck was very unroadworthy, it was desirable that we start on the east coast because we had some of the worst mountains in northern Korea to cross.

We managed to cross these mountains to the east coast and went down the coast where we made our first survey of a division in training in the Unit Training Center. We then proceeded north to survey one ROK division in reserve, then proceeded up to the main line of resistance to survey two ROK divisions in the line. We then went back to Seoul and proceeded to the southwest part of Korea where there was another replacement training center where we surveyed the troops. We then were ordered to Koje-do Island to survey the North Korean prisoners of war, and then to Chedju-do Island, southwest of the mainland, where we surveyed the Chinese prisoners of war and then returned to Seoul.

We thought we had completed our job at this point, but since we had completed our first survey, certain changes in the ration system of the ROK Army had been instituted and Eighth Army wanted us to return to the western front to a division in the line to see whether there was any improvement in the nutritional status of the troops.

We had been doing this for months now, and one would think it would be easier, that we had fallen into all the pitfalls anybody could fall into, but that wasn't the case. We went up to one of the regimental command posts which was dug out against the hill with just one small window and door to let in ventilation. Since this was in July, the weather was particularly hot and humid. I told the commanding officer, after the preliminaries were over, I wanted to get his records to show exactly what his regiment received in the line of food for the last month.

He gave orders to his junior officers who came in with heaps of books, then sat down and started thumbing through them. They came up with a figure and indicated that this was what I wanted. I did a lot of mental gymnastics by taking the hundreds of thousands of kilograms of rice, dividing them by the strength of the regiment, then by 30 days, and came out with a ridiculous figure.

I told the interpreters that it was all wrong, and they went through the whole rigamarole again. Then the officer gave orders and a couple more came in with another stack of books. This was repeated with little more success.

By that time, 15 or 20 officers had crowded into the hut which was stifling hot, and I was suffering from the heat and humidity. I told the interpreter, "Get half of these men out of here or I will die right now from heat exhaustion." He swung his arms around, and they scattered like chickens. Only one left the hut, however, and he came back with a bamboo fan and stood behind me, fanning me.

I concentrated on rice issue and finally located the correct accounts. As I went down the list, I came to a word which they didn't know and I didn't know. They thumbed through a Korean-English dictionary and found the English equivalent which I had never heard of. Then they ran out and came in with a fish and put it beside me so I could tell whether it was fresh or dried or smoked. That continued with various food items until the atmosphere was almost unbearable. We were getting along fine until I came to one pen scratching which indicated two units of something per regiment. I asked, "Two what?"

They didn't know what it was, but it was two. So, after a conference, they decided that it was soy sauce.

"Two what? How much soy sauce?"

They went into another huddle and came up beaming all over. "Two grams." I told them that this was ridiculous because I knew that each soldier consumed much more than that.

They gave me a sour look and went into another huddle and after half an hour, they beamed all over and came back, "Drams, drams."

I felt like blowing my top and told them that they were getting closer but the answer was still ridiculous.

They looked at me as if I were the most stupid person in the world and went back into another huddle. After a while they came back beaming and said, "Drums, drums."

I asked, "What kind of a drum?" They replied, "A 55-gallon drum." I had them take me out to show me an example of one, and there, sure enough, was a 55-gallon drum of soy sauce. It was the most soy sauce I ever saw in one place in my life.

But, I wasn't the only one who had difficulty. Dr. Sandstead had been training his interpreter for a long time and was proud of him and wanted to put him to the acid test. We came to a field hospital where they had a lot of dysentery. Dr. Sandstead questioned the diagnosis and sought to determine the etiology through his interpreter.

He called the interpreter, a major M. C. of the ROK Army, and said, "I want you to find out what the symptoms are. Do they have pains? Fever? Are they passing blood? Exactly what is it?" We went into a great deal of detail, and the ROK major said, "Yes, sir," and disappeared. He had a conference with the hospital commander his surgeons and medical men for about an hour or so, and came back beaming. He said, "Colonel, sir, this man comes from Taegu."

So, as I said before, it wasn't easy.

(Dr. Sandstead gave his address entitled, "Appraising Nutritional Status—Recent Experiences with Far East," which was illustrated with slides. This address was off the record.)

V. Evaluation of Military Rations by Animal Experimentation

FRIDAY MORNING SESSION

26 February 1954

Dr. Wendell H. Griffith, *Presiding*

Chairman GRIFFITH:

I have pride in the achievements that have been made through our research program and particularly with reference to military feeding. And also, a great deal of chagrin; chagrin that apparently simple questions can be asked for which good answers other than opinions cannot yet be given. This is perplexing as we are here as a group of interested people with certain knowledge and experience in order to help the military in a problem which is far from being a simple academic problem. Then, there is a very real and practical problem, too. It is worth remembering that we are not dealing with matters that may be of concern in the future only. Decisions are being made today on the basis of incomplete evidence, but decisions have to be made and we should realize that as we consider these problems, that the quicker answers can be obtained, the better the feeding will be, not only in the Armed Forces, but in population groups generally.

I would like to emphasize one additional point. We hear frequently that the interest of the military may be in proper feeding for wound healing, proper feeding for resistance to disease—those are the two most commonly mentioned. I think we should remember that those are only somewhat isolated instances of the application of just plain, ordinary, good nutrition, that we must understand normal nutrition before we can really do anything about abnormal nutrition, and we are concerned then with what it takes to keep one physically fit, and that emphasis should only be in our minds.

Yesterday, we had the evaluation of methods for the evaluation of proteins, of vitamins, of minerals. Today, we are concerned with the question of the evaluation of these substances put together in the form of rations and the effect of foodstuffs on people. These are not academic questions. These are very real questions. These are rations that are being prepared today and used today, and the people who are eating these rations are not paper people, they are real people. The responsibility that we have is one that deserves our most serious consideration and our planning, in the hope that we may arrive at what the engineer would call the proper relationship between what goes into the machine, the body, and what we get out of it.

We lose track sometimes, in our interest in let's say wound healing or other aspects of disease, not only of the very important matter of the work output which we can get from a certain expenditure of energy and supply of not energy itself, but of food materials. We need a little more of the engineer's point of view here. The engineer is not satisfied with a 95 percent performance with a gasoline, if he can get a 97 percent performance. We should not be complacent about that type of improvement, because that is part of the improvement that will lead us to what we may call an efficient use of the foodstuffs in good nutrition.

Rats

HARRY SPECTOR

Nutrition Division, Quartermaster Food and Container Institute for the Armed Forces

Introduction—Need for Biological Evaluation

Tactical situations, climate, terrain, and feeding methods and equipment create a large number of feeding problems which are met by a number of different rations, supplementary packs, and food packets. For troops subsisted on A (garrison type) or B (overseas field type) rations, the problems of adequate nutrition are not much different from those encountered in any type of mass feeding. But in all combat phases of military operations the maintenance of physical fitness through adequate nutrition is a complex and difficult problem.

The difficulties in attainment of nutritional adequacy for *combat* or packaged operational rations stem from the need to incorporate other functional qualities which are necessary under the circumstances of their use to fulfill their purpose in the field. These other essential requirements or *military characteristics* are acceptability, stability, and field utility (7).

Army Regulation 40–250 dictates the minimum allowances of nutrients which a ration must supply in order to be considered adequate. These basic standards of diet (table 1) for the Armed Forces prescribed by the Surgeon General are patterned after the recommendations of the National Research Council, but modified in the light of previous experience to meet the needs of the Army. In normal civilian feeding the recommended allowances can be attained with a good variety of common foods which will also provide other minerals and vitamins for which requirements are less well known. It is not safe to make this assumption for packaged rations since they consist largely of processed foods. Indeed, investigations with World War II rations

(*4, 5, 8, 9*) have alerted us to the fact that packaged rations may be physiologically deficient in nutrients despite being apparently adequate by calculated comparison with nutritional standards.

TABLE 1

BASIC DIETARY STANDARDS.

	Energy	Protein	Iron	Calcium	Vitamins				
					A	C	Thiamine	Ribofl.	Niacin
	Cal.	gm.	gm.	mg.	I.U.	mg.	mg.	mg.	mg.
N.R.C. Recommended Allowances Physically Active Man (Oct.'48)	3,000	70	12.0	1,000	5,000	75	1.5	1.8	15.0
Army Minimum Allwances AR40-250 (Oct.'47)	3,600	100		700	5,000	50	1.6	2.2	16.0

Field trials are conducted by the Medical Nutrition Laboratory of the Surgeon General's Office to determine the nutritional adequacy of military rations under actual or simulated conditions of use. These trials with men in the field are undeniably the ultimate test of the adequacy of military rations. The cost in manpower, time, and money is so high, however, that many years frequently intervene between trials of a specific ration.

Ration development is in a constant state of flux due to the needs of modern mobile warfare on a global scale. A relatively rapid biological tool is therefore required (*a*) to check the nutritional adequacy of rations while they are in the course of development, (*b*) to determine necessary corrective measures where nutritional inadequacies may be found, and (*c*) to determine the nutrient stability of such rations. For this reason and those previously stated, it is considered important to devise and conduct feeding tests with laboratory animals to ascertain the physiological availability of the nutrients in packaged rations.

Animal Experimentation as a Tool

The limitations or reservations associated with a particular technique are usually related last by their advocates. However, a candid discussion of these at the outset is advisable to establish the usefulness of animal experimentation in general, and rat experimentation specifically, as a tool within the framework of appropriate reservations. Most conspicuous of these general reservations is that of species differences between laboratory animals and man in their nutritive requirements. Quantitative more than qualitative differences complicate interpretation of information secured with laboratory animals for the needs of man. Quantitative differences are, furthermore, confused by different systems of expressing nutritive requirements; i. e., amount (*a*) per day, (*b*) per unit of diet, or (*c*) per unit of body weight. In spite of this, much of our basic knowledge of human nutrition was first established with laboratory animals. Utilizing a variety of laboratory animals helps to clarify the significance of species differences where previous knowledge of these may not be available.

Turning specifically to the rat, its ability to synthesize ascorbic acid does not limit its usefulness for evaluating packaged rations inasmuch as fortification accounts for almost all of this vitamin present. Due to intestinal synthesis of folic acid and biotin by the rat, dietary deficiencies of these two vitamins cannot be demonstrated without resort to sulfa drugs. In view of the limited requirement for these vitamins in human diets, this may not be an unfortunate circumstance.

The growth response of weanling male rats affords a simple, rapid, and economical criterion for screening packaged rations for possible nutritional inadequacies since all essential nutrients have a growth impact when not present in adequate amounts. This is particularly true of the more labile nutrients, the amino acids and vitamins, which are therefore more likely to be inadequate in packaged rations.

Systematic study of the nutritional adequacy of combat rations by animal feeding experiments was first conducted at the University of Wisconsin by Elvehjem and his students (*5, 8, 9*). They showed that young monkeys, dogs, and rats failed to grow normally when fed such rations as K, 10–in–1, and C_2 as the only source of food. Their early rat studies established the following general outlines which have served as a base line for detailed studies of more recent rations: The addition of B vitamins and casein to the rations substantially improved growth in all cases—attaining a normal rate when K and C rations were used. Supplementation with vitamins A and D or salts IV had no effect upon the growth of rats fed any of the combat rations. More recent studies at the Quartermaster Food and Container Institute will now be reported as examples of application of rat growth as a means of evaluating the nutritional adequacy of combat rations.

Methods

The general techniques used in preparing the rations for rat feeding experiments are as follows:

1. *Diet preparation.*—The components of all menus of a ration are first ground and mixed in a large Hobart mixer. They are then thoroughly blended in a large Turmix Food Mixer.

2. *Analysis.*—The composited ration is then analyzed for proximate composition, calcium, iron, vitamin, and essential amino acid content.

3. *Supplementation.*—Supplements of protein, a mixture of all the B vitamins, and specific amino acids or vitamins suggested by the ana-

lytical results are added individually and in various combinations to the composited ration.

4. *Storage of ration.*—The daily food requirement for each experimental group (five rats) is packaged separately in polyoxyethylene bags and appropriately labeled. These bags are frozen, stored in the frozen state, and allowed to thaw out in the refrigerator overnight for the next day's feeding. This technique makes it possible to make one uniform mix of a ration to last for the entire experimental period of 6 weeks without complication from mold growth. Due to their high-moisture content, storage of packaged rations, even in the refrigerator, usually results in the growth of mold over a 2-week period.

The multiple range test of Duncan (*1*), a significance test for differences between ranked treatments in an analysis of variance was used to analyze the results. This test acts as a homogeneity-of-means test like the F test for the determination of the significance of differences among means; in addition, it gives decisions on the relative merits of the treatments considered in all possible pairs. The multiple range test is consequently superior to the F test which does not indicate which of the differences among the means may be considered responsible for rejection of the homogeneity hypothesis. Moreover, if the 2-sided *t* test were applied to more than 2 means, the error probability would increase with the number of means considered. For example, the error probability at a 5-percent level could be as much as 20 percent for 4 treatments and as high as 91 percent for 20 treatments. In Duncan's multiple range test the levels based on degrees of freedom incorporate a correction for the number of means compared to give consistent levels of significance.

Moreover, the graphic manner in which this test is performed makes it possible to interpret voluminous data in a relatively short time and to clearly sight significant results which might otherwise be obscure. Thus, the differences between all pairs of means enclosed in a common bracket are not significant. All other differences between single means are significant. It should be pointed out that when differences between means are found to be significant by the multiple range test they would always have been found to be significant by the 2-sided *t* test at the same level of significance. In addition, when it is desired to compare only differences between single means and not to test linear comparisons within several means, the multiple range test is not appreciably less powerful than a multiple F test and requires considerably less time and effort.

Results of Rat Growth Studies and Their Analysis

Examination of the data (table 2) on average growth at the end of the fourth week for rats fed the Small Detachment Ration, 5-in-1,

TABLE 2. EFFECT OF SUPPLEMENTS ON THE GROWTH OF RATS FED THE 5-IN-1 B RATION

Group No.	Casein 5%	Amino Acids•	B Vits••	4-week Period Gain/week gm. .01 / .05	Group No.	6-week Period Gain/week gm. .01 / .05
3	+		Mix	A 41.6 A	5	A 40.1 A
5	+		B6	41.4	3	39.7
4	+		B_6 + PA	39.3	4	B 38.2 B
2	+			C B 34.4 B	8	C 34.8 C
8		M+Ph		34.0	2	34.1
6	+		PA	33.0	6	32.6
7		M Ph T		D 31.3	10	31.4
10		M T		30.8	7	30.5
9		Ph T		E 24.8 C	9	D 24.9 D
1*				20.5	1*	21.8
				S.E. 1.62		S.E. 1.31

* Unsupplemented 5-in-1 B. Protein 7.94% (15.57% dry basis), Calories/gm. 2.57 (5.04 dry basis).

• M = Methionine 0.15% Ph = Phenylalanine 0.10% T = Tryptophane 0.05%

•• Vitamin Mix supplied the following levels in milligrams per 100 grams of ration: thiamine HCl 0.25, riboflavin 0.50, pyridoxine HCl 0.25, niacin 2.0, Ca pantothenate 2.0, inositol 10.0, p-amino benzoic acid 5.0, folic acid 0.1, Choline 100.0

Arctic Trail Ration (Haliver Oil Orally) 37.4±2.30 35.0±0.69
Protein 21.38% (22.32% dry basis)
Calories/gm. 5.01 (5.23 dry basis)

using the 0.01 level of significance leads to the following major conclusions:

[E] The combined addition of phenylalanine and tryptophane (**9**)[1] to the 5-in-1 B ration did not improve the growth response.

[C] Casein (**2**) was beneficial.

Methionine, however, produced a growth response equal to casein.

Phenylalanine and tryptophane did not augment the response from methionine.

Pantothenic acid (**6**) gave no response above casein.

[A] The B-vitamin mixture when added to the casein-supplemented ration (**3**) gave a significant response above that of casein alone (**2**).

Pyridoxine (**5**) gave a growth response equivalent to that produced by all the B vitamins. This is particularly interesting since microbiological assay indicates that apparently adequate amounts of this vitamin are present in the ration.

The Arctic Trail Ration, a concentrated ration with a relatively high level of protein, produced a very good rate of growth in rats when only haliver oil was given as an oral supplement. Haliver oil was given since the first version of this ration furnished very little vitamin A.

The Individual Combat Ration C is the principal ration used by combat troops when they have been denied the use of their unit kitchens (table 3). Examination of the data on average growth at the end of the fourth week for rats fed the C–4 ration using the 0.05 level of significance leads to the following conclusions:

[H] Urea and ammonium citrate as nonprotein sources of nitrogen fed at levels equivalent to 5 percent and 10 percent casein did not improve the growth response. In fact, the higher level of ammonium citrate was toxic. In other experiments, a mixture of the nonessential amino acids fed at levels equivalent to 5 percent and 10 percent casein was without benefit, and 5 percent glycine depressed the growth response.

[F] When vitamin B_{12} was added alone (**2**) improved growth was obtained. The unsupplemented C–6 ration (**23**) promoted a greater rate of gain in rats than did the C–4 ration.

[D] One-tenth percent methionine (**3**) is in the same bracket as 0.1 percent methionine plus folic acid plus B_{12} (**5**), 0.3 percent methionine plus B_{12} (**12**), 5 percent casein (**17**) and 10 percent casein (**13**), which indicates that (*a*) 0.1 percent methionine is adequate to correct the methionine deficiency, (*b*) B_{12} exerted a sparing effect on the limited methionine present but had no beneficial effect when the methionine was made adequate, (*c*)

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[1] Bold-faced figures refer to experimental group numbers (see tables 2, 3, 4, 5).

TABLE 3. EFFECT OF SUPPLEMENTS ON THE GROWTH OF RATS FED THE C-4 RATION

Group No.	Casein %	Meth. %	B Vits.	Urea %	NH_4 Citrate %	4-week period Gain/week gm.	Group No.	6-week period Gain/week gm.
15	10		Mix			40.1	15	37.1
16	10		Mix B_{12}•			39.0	16	35.1
7		.1	Mix B_{12}			35.6	7	33.8
8		.1	Mix			32.3	17	32.0
9		.2				32.3	8	31.7
17	5					32.3	10	31.4
10		.2	B_{12}			31.8	9	30.6
11		.3				30.4	12	30.6
4		.1	B_{12}			30.0	11	29.9
12		.3	B_{12}			30.0	13	29.5
14	10		B_{12}			29.7	14	29.5
5		.1	FA•• B_{12}			29.6	4	28.7
13	10					29.6	3	27.4
3		.1				28.5	5	27.3
6		.1	FA			25.3	6	25.4
23**						24.0	23**	24.4
2			B_{12}			23.4	2	23.5
22				1.5		20.6	21	21.7
21				3.0		20.0	22	21.6
20					5.65	18.7	20	21.5
1*						18.2	1*	18.8
19					11.3	13.8	19	14.4
						S.E. 1.40		S.E. 1.46

* Unsupplemented C-4 Protein 7.00 (17.02 % dry basis) calories/gm. 1.91 (4.65 dry basis).

** " C-6 " 7.04 (16.05 % ") " " 2.05 (4.68 " ").

• Vitamin B_{12} 0.05 micrograms per gram of ration.

•• Folic Acid 1.0 " " " " "

folic acid also exerted no effect, (*d*) the major effect of the casein addition is due to the methionine supplied.

[A] At the 0.01 level shows that the addition of the other B vitamins with 0.1 percent methionine (**7**) gave a significant improvement over methionine alone (**3**).

Ten percent casein plus the B vitamins (**16**) was no better than 0.1 percent methionine plus B vitamins (**7**). Other experiments have established that pyridoxine accounted for most of the growth improvement with the vitamin mixture.

The highest ranking groups decreased continuously in average weekly gain from the fourth to the sixth week. The lowest ranking groups increased from the fourth to the fifth week, and the sixth week while less than the fifth was higher than the fourth week. These observations indicate that the C ration was deficient during the most rapid stage of growth of the rat. Once this stage was past the C ration became relatively less deficient and the beneficial effect of the supplements diminished.

The September 1951 assembly of the C ration, the latest one tested, is representative of the largest procurement of this ration made for use in Korea. Eighty percent of this ration was used as a basal (table 4). When protein supplements were used they were added in amounts to make all diets isonitrogenous. Sucrose and corn oil were then added in amounts to make all diets isocaloric.

Examination of the data on average growth at the end of 3 weeks of controlled feeding using the 0.05 level of significance leads to the following conclusions:

[E] The liver concentrate (**8**) did not improve growth.

[D] One-tenth percent methionine (**10**) gave a growth response when the protein level was only 11.19 percent equivalent to that obtained when the level was increased to 19 percent with alpha protein (**2**).

[A] [B] [C] Show that addition of 0.1 percent methionine to alpha protein (**3**) gave a significant improvement in growth, contrary to the results when casein was used as a protein supplement, probably because of the low methionine contribution to alpha protein (0.045 percent). Addition of vitamins or liver preparations to this combination was of no value. This indicates that this new C ration is adequate for the rat in all the known B vitamins and unidentified factors contained in desiccated liver. The slight improvement when the higher level of desiccated liver was added alone (**7**) was probably due to the methionine contributed (0.13 percent).

The data from the subsequent 3 weeks of *ad libitum* feeding are in general consistent with the data on controlled feeding with the following exceptions:

TABLE 4. Effect of Supplements on the Growth of Rats Fed the C Ration (September 1951 assembly).

Group No.	Alpha Protein* %	Meth. 0.1%	B Vits.	Liver** %	Controlled Weeks 1-3 Gain / Week gm. .01	Controlled Weeks 1-3 Gain / Week gm.	Controlled Weeks 1-3 Gain / Week gm. .05	Group No.	Ad Lib Weeks 4-6 Gain / Week gm. .01	Ad Lib Weeks 4-6 Gain / Week gm.	Ad Lib Weeks 4-6 Gain / Week gm. .05
5	2.26	+	Mix	3.22 D	A	26.6	A	5	A	45.1	A
3	4.52	+			B	25.3	B	4	B	43.5	B
4	4.52	+	Mix		C	24.5		6		43.0	
9	4.52	+	B6			23.3		9	C	38.5	
6	2.26	+	Mix	4.13 C		23.0	C	3	D	36.8	C
7				6.44 D	D	20.9		7		34.5	D
2	4.52				E	18.5	D	10		30.0	
10 *		+				18.4		2	E	19.5	E
1 *					F	15.7	E	8		17.5	
8				8.26 C		14.5		1 *		14.9	
						S.E. 0.77				S.E. 1.80	

* 80% of C ration providing 11.19% Protein (dry basis); all other groups provided 19.0% Protein (dry basis). All diets adjusted to 4.04 calories/gm. (dry basis).

• Peroxide treated to remove sulfites

• • Liver D = Desicated liver
Liver C = Liver Concentrate

Storage Conditions Temp.	R.H.	Length of Storage Months			Apparent Digestibility %	
°F	%	6	12	18	Fat	Protein
-20	40	31.8 ± 1.23	29.0 ± 0.90	27.6 ± 0.50	96.4	80.2 ± 0.92
70	50	28.8 ± 1.20	28.6 ± 1.81	29.3 ± 1.43		
100	90	28.7 ± 0.87	** 24.9 ± 0.87	** 21.3 ± 0.87	96.4	** 74.6 ± 0.67
Cycling*		30.0 ± 1.21	26.4 ± 2.45	* 22.5 ± 2.18		

* Simuloting desert conditions
12 hrs. 110°F 25% R.H.
12 hrs. 70°F 90% R.H.

*P = .008
** P = <.001

TABLE 5. Effect of Storage on the Growth of Rats Fed the C Ration (September 1951 assembly).

[E] Increasing the level of protein with alpha protein (2) was of no benefit. This indicates that methionine rather than the level of protein was limiting growth and confirms previous indications that the response from casein was due to the methionine contributed. The explanation for the significant effect obtained with alpha protein during the first 3 weeks on controlled feeding probably lies in the well-known higher requirement for protein immediately after weaning.

[A] The addition of B vitamins alone (4) and with desiccated liver (5) gave a growth response above that obtained by alpha protein and methionine (3). It is interesting to note that the growth response during the first week of *ad libitum* feeding was 61.2, 64.2, and 47.8 gm., respectively, whereas during the third week the growth responses were quite uniform, 27.4, 29.2, and 27.8 gm., respectively.

The effect of storage on the average growth of rats fed the 1951 assembly of the C ration for 4 weeks is shown in table 5. One year's storage at 100° F. resulted in a significant drop in growth-promoting ability, whereas storage under simulated conditions was not significantly detrimental until 18 months. The latter picture is complicated by the erratic operation of the cycling room. Microbiological assays revealed no significant drop in amino acid content. However, the protein digestibility after 18 months' storage at 100° F. was significantly less than when stored at −20° F.

Although losses of thiamine and pyridoxine were found on analysis, supplementation with these vitamins and the B-vitamin mixture did not compensate for the loss in growth response. Incidentally, the *ad libitum* consumption of 100 percent of this C ration gave the best growth response ever observed on this unsupplemented combat ration.

Evaluation of Other Criteria

Since much of the work on newer or unidentified factors is being done with rats, rat growth as a tool is continuously being brought up-to-date. After screening by growth studies has established specific nutrient deficiencies, further quantitative refinement is possible by one or more of the following criteria: (*a*) blood levels, (*b*) balance studies, (*c*) enzyme activity, (*d*) carcass analysis or any of the other functions mentioned yesterday. For example, several recent studies in Dr. Elvehjem's laboratory have been concentrated on the metabolism of vitamin B_6 (*3*). It was demonstrated that the mixture of the forms of vitamin B_6 found in the K ration was not significantly less active than pyridoxine when added to a sucrose ration and could not be considered responsible for the increased need for the vitamin. Excretion of relatively low amounts of vitamin B_6 in feces of rats fed the K ration with and without vitamin B_6 has indicated that intestinal synthesis does not occur to the same extent on the K ration as it does on a dextrin diet. The vitamin B_6 content of the liver was significantly lower in rats on the K ration not supplemented with this vitamin and represented 50 percent less than that found in the normal weanling rat liver.

Rats are quite tractable under conditions simulating the military stresses of climate and heavy work. The resistance to climatic stress can thus be used as a practical measure of the nutritional adequacy of combat rations. Forced activity can be applied (*a*) in fixed amounts as a measurable imposed physical stress, using other criteria, as growth, to determine dietary adequacy; or (*b*) to exhaustion of the animal as a direct measure of nutritional adequacy for severe physical work. These criteria provide an indication of dietary adequacy in terms of optimum requirements for military stress conditions.

Ability to support reproduction and lactation in rats was one of the first criteria applied to estimate the nutritive value of the combat rations. The following results obtained with 10 females fed the C_2 ration (*2*) are cited as representative: "5 reproduced and lactated normally, 3 reproduced but ate their young soon after birth, 1 did not become pregnant, and the other one developed a respiratory infection and was sacrificed without mating." Although reproduction and lactation represents a greater nutritional stress than does even growth, the significance of this higher requirement by the female rat as a measure of the adequacy of rations for the combat soldier is obscure. Since the requirements for reproduction in the rat are less well defined than for growth, and other than nutritional factors may play an important role, it is frequently impossible to obtain a statistical comparison of the effects of different diets. These uncertainties coupled with the more tedious and time-consuming routine involved in reproduction studies argue against this criterion.

Summary Appraisal of Feeding Studies With Rats

A practical appraisal of the significance of feeding studies with rats for evaluation of the nutritional adequacy of combat rations for military personnel yields the following perspective summary of advantages and limitations. The growth response of weanling male rats fed a composite of all menus of a ration for 4 weeks serves as a simple, rapid, and economical criterion of overall nutritional adequacy. Specific nutrient deficiencies can be established by appropriate supplementation. The ability to achieve homogeneity in the test animals and to control all pertinent variables makes it possible to establish statistically significant differences with only five rats per group. Rat growth studies can reveal physiologic inadequacy of nutrients not ascertainable by chemical, physical, or microbiological assay.

It is well established that the amounts of vitamins and amino acids required for growth are greater than for maintenance. In "pilot" testing the quality of the food furnished our combat troops it is not unrealistic to apply the higher nutritional standard represented by growth since in a draftee Army a significant segment may be in the stage of rapid adolescent growth.

Our rapidly increasing knowledge of species differences is improving our ability to interpolate the results of rat growth studies to man. For example, the methionine content of the C ration while manifestly too low a concentration for the growing rat (0.313 per cent, dry basis) furnished 2.27 gm. per day, which we know satisfies Rose's (*6*) liberal Recommended Allowance of this amino acid for man.

The nutritional needs of man are not as fully understood as those of rats. Deficiencies which can be readily demonstrated in rats may not be recognized in man by present methods of appraisal, yet may cause lack of stamina, decreased efficiency, diminished resistance to disease, and may delay recovery from wounds. Rat feeding studies can provide guiding information on such marginal deficiencies. Field trials in which performance is measured and the psychological aspects are introduced will permit us to ascertain the significance of these pilot tests for the nutritional welfare of members of the Armed Forces.

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Nutrition and Performance in Animals [1]

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All who have had a direct contact with the experimental study of human nutrition are fully aware of the difficulties involved in such an undertaking. They will agree readily that it is advisable to obtain all the relevant information that can be obtained from animal studies before taking up the costly and not infrequently dangerous experimentation on man. At the same time, the limitations of applicability to man of conclusions based on animal nutritional research, in general, and of the researches on performance, in particular, should be kept in mind.

In the past, growth and health have been used most frequently in animal studies as criteria of nutritional adequacy of human diets, including military rations. As a rule, "growth" meant increments in body weight, although carcass analyses have been used occasionally and blood analysis of certain nutrients, capacity for reproduction and lactation, and resistance to nonnutritional stresses have been also examined.

It is the purpose of this communication to point out the potential contribution of studying performance as a criterion of nutritional status. It is our belief that opportunities afforded by the techniques for the study of animal behavior have not been fully exploited in the interest of nutritional research. To the best of our knowledge, there is not a single nutritional laboratory in which the problem of animal performance in relation to dietary factors has been or is being studied systematically. This is one of the areas which perhaps can be cultivated most effectively by combining personnel competent in the biochemical and the behavioral sectors of animal biology. Most of the research in this field has been done by animal psychologists for whom diet is just one of a large number of factors influencing behavior. In much of their research, these investigators have been concerned with other aspects of behavior than "performance," such as activity motivated by nutritional requirements (hunger cycle, food selection), and food hoarding. Hunger and thirst are frequently used as the basic drives for behavior in the study of a variety of problems which have no direct or even indirect interest to the nutritionist.

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In this connection, I should like to draw attention to Munn's *Handbook* (*6*) of animal psychology, based on the researches on the rat. The volume provides a thoroughly documented, up-to-date survey of facts and theories concerning the various aspects of unlearned and learned, individual and social behavior of the most intensive studied mammal. The *Handbook* represents a ready reference for a large number of test procedures, applied but frequently not adequately described in current experimental studies, and it helps to reduce the frustration of the nutritionist who wants to read with full comprehension the animal psychologist's writings. This is not a nutritional handbook, organized in reference to the nutritionist's criteria, but it reviews a good deal of the literature on nutrition and animal behavior.

From the point of view of performance capacity, four aspects of behavior are particularly interesting:

(*a*) *Learning* (*running dry and water mazes, acquisition of conditioned responses*) *and problem solving* (*6*, *7*).—While in the fundamental studies intellective capacity is an important aspect of the animal's overall "fitness" and its partial dependence on diet an important fact, in the applied research on the evaluation of military rations this criterion may not be particularly useful or relevant.

(*b*) *"Voluntary" locomotion, as measured by the revolving-drum technique* (*6*).—Krzywicki and Da Costa (*3*) observed an average activity of 1,101 rotary cage revolutions per rat per day in a control group of animals fed *ad libitum*, with an average daily intake of about 15 gm. of food. Eighteen rats, fed a quantitatively restricted diet (intake of 8 gm. per day), averaged 7,854 cage revolutions per day and their spontaneous activity decreased to or below the prerestriction level during the period of refeeding. Qualitative restrictions of the diet also frequently result in higher activity levels (*1*). Care must be taken in the interpretation of the data (*8*). It is quite clear that in these situations the increased activity is not a reflection of an increased "vitality" and work capacity.

(*c*) *Endurance.*—This important aspect of behavior has not been considered by Munn. A method for recording sustained performance in swimming rats was applied in a study on the influence of thiamine deficiency by Kniazuk and Molitor (*2*). The technique appears preferable to forced performance to exhaustion on the treadmill, as it completely eliminates the possibility of mechanical injury. Attaching different weights, carried by the swimming animal, provides a simple way for varying the workload. The authors used the test for measuring either endurance or peak performance. In the latter case they started with light load and increased it rapidly. Within 1 to 2 minutes the maximal weight was reached which the animal was able to carry without sinking. In testing endurance, the animal is made to carry a submaximal load, swimming either to exhaustion or until it drops to a specified distance below the starting level. The two aspects of capacity for hard muscular work are not equally sensitive to different stresses.

(*d*) *Sensory functions* (*6*).—In this context, Morgan's study of dark adaptation in normal and in vitamin A deficient rats (*4*, *5*) is of particular interest as it has demonstrated the possibility of a quantitative analysis of this sensory process in the rat. The discriminatory performance involved pressing of a lever located below the brighter 1 of 2 patches. The correct response was rewarded by a bit of food while the incorrect response was followed by an electrical shock. As an example, with a given patch brightness (3 log micromillilamberts) the control animals reached the 75 percent level of correct responses after 15 minutes spent in the dark while the corresponding time in the depleted animals was about 3 times as long. Pooling the data obtained for different brightness levels the author was able to draw dark adaptation curves for the control and experimental animals. The curves were similar in shape but the brightness of the test patch, at which the animals discriminated accurately between the dark and the illuminated patch after a given time spent in the dark, was substantially higher for the experimental animals than for the rat receiving a supplement of cod liver oil.

In summary, the study of performance in animals deserves more attention from the nutritionist than it has received in the past. It is in the area of endurance testing, an area which is difficult to explore in man, that animal studies may offer research tools particulary relevant and valuable for the evaluation of military rations by animal experimentation.

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Monkeys and Dogs

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The extensive developments in the field of nutrition have allowed us to measure most of the individual nutrients found in foodstuffs on a quantitative basis. In spite of this extensive knowledge, it often becomes necessary to use animals to test the availability of individual nutrients or to test the overall effectiveness of food combinations. Military rations fall into the latter class. There is always a question regarding the best animal to use for such tests. I think all of us agree that the rat is the most suitable animal because of our long experience with this species, unless there is a definite reason for its limitation. Experience tells us that there have been such limitations but as further information has been gained it has been found that the rat can be used under proper conditions. The rat was of no value in early work on the antipellagra factor, but when the relation of niacin to tryptophane was established, it soon became possible to produce a condition in rats which responds to the administration of niacin. During the early studies on the vitamin-B complex we often wondered why we could not produce a pernicious anemia-like syndrome in the rat. Today we know why; namely, casein carries appreciable quantities of vitamin B_{12}.

When we find rats unsuitable, we usually turn to the chick, the dog, and finally the monkey. Some might suggest that the monkey be given priority because it most closely resembles the human being. This may be true with certain nutritional studies, but I doubt that we can generalize very far. I might add that we started work on monkeys as a result of studies dealing with poliomyelitis. We immediately recognized that very little was known regarding the quantitative nutritional requirements of this species. We do not have time here to outline these early studies. It is only necessary to state that the vitamin requirements of the monkey are not greatly different from those of the rat except that a deficiency of certain of the B vitamins is produced more readily, especially folic acid. It also appears that the monkeys require an unidentified factor necessary for the maintenance of a normal blood stream, especially a normal neutrophillymphocyte ratio. More recent work has shown that large amounts of folic acid and vitamin B_{12} seem to reduce the dietary requirement for this factor.

Dr. Spector has pointed out that the 5-in-1 Ration appears to be adequate for supporting satisfactory rates of growth in young rats. In order to obtain additional information about this ration, we have fed it to monkeys. Our animals were obtained directly from dealers although a few were those that had been in our laboratory for some time and had received a synthetic ration containing an adequate quantity of all the vitamins. The components of the ration were removed from the containers and mixed in a Hobart mixer. The mixtures were kept in a cold room and were usually mixed fresh about once a week. All the ingredients were included except the soluble coffee product. Case histories of two typical animals will be presented.

Monkey AR–17 thrived for about 4 months on the 5-in-1 Ration and then began rapidly to regress, soon losing interest in food and holding to the side of the cage for support. Swelling around the eyes was the most obvious symptom other than general weakness. Since this condition suggested a possible thiamine deficiency, this vitamin was added at five times its normal requirement for this animal. No improvement was noted with this vitamin but a supplement of ascorbic acid brought about a rapid weight gain. It should be pointed out that the soluble coffee product was not included and that this powder contained supplementary ascorbic acid. It was not surprising, therefore, that the monkey would show an ascorbic acid deficiency. After the ascorbic acid had been administered for 1 month, a second deficiency began to develop. A decline in weight of 4 months duration was successfully reversed by a combined supplement of pyridoxine, inositol, folic acid, and the ascorbic acid. Further work indicated that inositol could be omitted from the mixture but that the others, especially folic acid and ascorbic acid, were needed. Blood analyses on this animal indicated a normal hemoglobin level even after being on the experiment for 1 year. During the administration of only folic acid and ascorbic acid, the hemoglobin value declined to a low of approximately 5 gm. percent. The erythrocyte and total leukocyte counts, as well as the differential leukocyte counts, remained normal throughout the experiment. Although the supplements were increased to include pyridoxine, calcium pantothenate, and finally thiamine and vitamin B_{12}, the hemoglobin did not rise above 8 gm. percent during the remainder of the experiment. When this animal was shifted to a synthetic ration the hemoglobin returned to normal over a period of a few months.

Monkey AR–51 was a male animal that had been in our laboratory for some time. It previously received a purified ration supplemented with vitamins and had at times been given extra foods such as bananas, apples and peanuts. This animal grew at a satisfactory rate for about 8 months while given the 5-in-1 Ration plus ascorbic acid. Following a 2-month plateau in weight a serious decline occurred. When the animal had lost about 500 gm. and had lost an interest in food, folic acid supplementation was initiated. Although the vitamin solutions were readily consumed, no improvement in the animal's condition resulted. Five doses of procaine penicillin G totaling 440,000 units were also administered during a 2-week period without apparent benefit. In an attempt to save the monkey's life without giving a complete vitamin mixture, pyridoxine was also added to the supplement. As a result of this supplement an almost immediate growth response occurred. After 2 weeks of weight fluctuation while tissue reserves of the missing nutrients were being replenished, a regular and quite rapid return to normal weight occurred. The animal continued to increase in weight during the remainder of the experiment while receiving this supplement.

Blood data obtained during the study showed a normal hemoglobin value of 12.2 to 15.3 during the entire experimental period. A differential white cell count made when folic acid supplementation was initiated indicated a slight reversal in the neutrophil-lymphocyte ratio. This symptom has been shown previously to be a characteristic of the monkey anemia factor. After the animal's recovery the neutrophil-lymphocyte ratio returned to normal. Total leukocyte and erythrocyte levels were not abnormal even while the animal's condition was the weakest. We may summarize then by saying that the 5-in-1 type of combat ration is capable of maintaining a state of health in monkeys for periods of several months depending upon the reserve supply of nutrients which the monkey has at the time the experiment is started. The two most limiting factors are folic acid and pyridoxine. If these are added along with ascorbic acid, the monkey can be maintained for periods of 2 years or more. The folic acid inadequacy is largely a reflection of the monkey's high requirement for this nutrient. The need for pyridoxine is another manifestation of the extra need of pyridoxine when highly processed rations are used.

The results with the C rations are not as conclusive because experiments have not been carried for as long periods of time. However, again I shall give case reports on five animals which have been fed this ration.

Monkey AR–51 had been used for studies on the 5-in-1 Ration and was about 2½ years old when it was placed on the C ration. Initial weight was 3,400 gm. and the hemoglobin content of blood was 14.5 gm. percent. This animal maintained its body weight for 2 months. After this time the animal showed a decrease in activity, a slight anorexia, an alopecia, and a graying and unkempt appearance of the fur. Severe anorexia occurred by the end of the third month resulting in a decline in body weight which became critical. At this point various combinations of B vitamins were administered, biotin, calcium pantothenate, and pyridoxine were given at five times the daily requirements. No response occurred and this original supplement was followed by additions of folic acid and thiamine and riboflavin. The animal did not respond to any of these vitamins and continued to lose weight. Other B vitamins were added as well as the fat-soluble vitamins but the response was negative in all cases, although after 5 weeks of this supplementation a small increase in weight resulted, the increase being from 2,640 gm. to 2,850 gm. Again the animal showed reduced activity and reduced interest in food. At this point vitamin-free casein was added to the ration at a level of 10 percent. The animal showed an immediate response and the growth continued until the animal reached 3,600 gm. At this point both the casein and vitamin supplements were removed from the diet and the animal has continued active and healthy since 11 January 1954. Actually within the past 3 weeks the animal has shown considerable weight increase and now weighs 3,800 gm.

Animal AR–54 weighing 2,200 gm. was placed on the C ration 17, June 1953. The animal refused to eat the ration and lost 300 gm. body weight. The diet was supplemented with fresh fruits and vegetables until the ration became acceptable to the animal. On 4 July 1953, the animal was placed again on the C ration alone. The animal now gained weight and reached a weight of 3,200 gm. by the latter part of September or after a 3-month period. During October it maintained its body weight but showed a slight graying of the fur. The animal then developed severe anorexia and the body weight decreased to 2,300 gm. by 17 November 1953. A vitamin supplement consisting of 10 times the daily requirements of folic acid, thiamine, riboflavin, pyridoxine, and biotin was administered on 18 November, but the animal did not respond. The anorexia became so severe that it was necessary to place the animal on a purified diet supplemented with high levels of B vitamins and fresh fruit and vegetables. The animal responded slowly to this regimen and on 1 January 1954, was placed again on the C ration but B-vitamin supplements were continued. At the present time the weight of the animal is 3,200 gm. and it shows no severe deficiency. The hemoglobin level of this monkey was 13 gm. at the start of the experiment. It dropped to 10 gm. before the B-vitamin supplement was initiated and after supplementation the level returned to 13 gm.

Monkey AR–58 was received in the laboratory the first part of September 1953. It was given our regular synthetic ration for about 2 weeks. On 18 September when it weighed 2,400 gm. it was given the C ration. The animal consumed the ration but showed no increase in weight during the following month. The hemoglobin level of the blood remained at 14 gm. After about 6 weeks on the ration the animal developed a severe anorexia which was accompanied by loss of weight and lack of activity. After the animal had lost 400 gm., folic acid, pyridoxine, riboflavin, and calcium pantothenate were added. This supplement failed to correct the anorexia and it became necessary to give the animal fresh fruits and vegetables. The animal continued to lose weight, weighing only 1,800 gm. during the first week of November. After a week, noticeable signs of general edema were evident. The edema was not alleviated by giving an intramuscular injection of 25 mg. of thiamine. The edema condition improved slowly when 5 percent of casein was added to the ration. When the edema disappeared the monkey was placed on the C ration supplemented with all the B vitamins. The animal failed to increase in body weight and the hemoglobin dropped to 10.5 gm. At this point the monkey developed another severe edemic condition and died 27 November 1953. On autopsy no abnormal conditions were observable.

Monkey AR–17 was placed on the C ration on 17 June 1953. At this time it weighed 3,550 gm. and its hemoglobin level was 8 gm. percent. This low hemoglobin level was the result of the animal's extended period on the 5-in-1 ration. The animal maintained itself in fairly normal condition for a period of six months. The hemoglobin level varied between 8 gm. and 10 gm. and the weight increased to 3,970 gm. on 9 October 1953. During December this animal showed some anorexia with a steady decline in weight which reached 3,730 gm. on 23 December 1953. At this time the animal was given vitamin-free casein in his diet at a level of 5 percent, and folic acid, pyridoxine, C_a pantothenate, and biotin at five times the normal level. The animal responded with an increase in body weight up to 3,950 gm. The monkey was placed on the unsupplemented ration on 27 December 1953, and showed a rapid decline in weight. The diet was again supplemented with vitamins but no casein. The animal failed to respond to this supplementation as it had done previously. Six days later riboflavin was given and a few days later vitamin B_{12} was added. Only a slight temporary increase in weight resulted. All the vitamins were removed from the diet and for a period of several weeks it showed no change in body weight. At present the animal weighs 3,800 gm. and the hemoglobin content of the blood has increased to 11.6 percent.

Monkey AR–28 weighed 4,100 gm. and had a hemoglobin level of 9 gm. percent when placed on the C ration 17 June 1953. This animal showed a temporary increase in weight during the first month on the ration and reached a weight of 4,600 gm. During the following 5 months the monkey showed a steady but slow decline in weight accompanied by some graying of the fur. The hemoglobin level during this period remained between 9 gm. percent and 10 gm. percent.

A slight anorexia accompanied by a further decline in body weight and a decrease in activity occurred during December. The anorexia became quite severe during the fourth week of December and on 29 December 1953, the animal weighed only 3,730 gm. At this time the following vitamins: folic acid, C_a pantothenate, and pyridoxine HCl.

were added at 10 times the level used in synthetic rations. The anorexia disappeared completely on 9 January 1954, the animal weighed 4,200 gm. Although the vitamin supplement was continued, this animal lost 300 gm. during the following week. The animal continued to lose weight and a slight anorexia was observed. When inositol and PABA were also added to the vitamin mixture, anorexia disappeared and the animal reached a weight of 4,100 gm. on 20 January 1954.

The addition of inositol and PABA to the vitamin mixture was continued for 9 days. Although the animal showed an immediate response of 200 to 300 gm., its weight did not return to the previous level of 4,600 gm. Recently all the vitamin supplementation was removed and the animal showed a further gain up to 4,600 gm. It appears that some alteration in the microflora of the intestinal tract had taken place during the vitamin supplementation that tended to produce a favorable growth response.

Two dogs have been fed the C ration for the past 2 months. They were approximately 5 months old when placed on this regimen. During the first month 1 animal increased in weight from 7.9 K. to 10 K., and the other from 7.3 K. to 8.5 K. During the second month there was little change in body weight. This plateau in body weight was to be expected since they were reaching maturity. Further findings will determine if any deficiency develops. The hemoglobin level of the blood remained between 15 and 16 gm. percent.

Although our work on the C ration has not been as extensive as that on the 5-in-1, it appears that the deficiency is not as simple as that observed on the 5-in-1 ration. The animals do not accept this ration as well as the 5-in-1 and when they do show a decline in weight typical responses cannot be obtained by addition of pyridoxine and folic acid. The condition may be more complicated and may be due to disturbed digestive conditions. Since rats grow rather well on this ration, it appears that the combination of foods is not satisfactory for the monkeys. Since no specific deficiency develops, this ration may actually be as satisfactory for the human subject as the other rations. This combination may have quite a different effect in the tract of the human than the tract of monkeys. Furthermore, the ration is consumed under different conditions by human subjects, that is, the entire ration is not mixed, but certain components are eaten at each of the individual meals.

The Nutritive Value of Army Rations as Determined With Growing Chicks

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Recent research has shown the particular importance of adequate nutrition, especially in regard to the vitamins, in animals placed under conditions of stress. Fighting men oftentimes are forced to adapt to several different stress factors such as strenuous muscular work, extreme cold, and emotional disturbances simultaneously. Therefore, it appears desirable that the stress of inadequate nutrition be eliminated insofar as possible.

Since the requirements of the chick for the various vitamins have been fairly well established, this animal is useful in assessing the physiological adequacy of the vitamin content of a ration. Feeding studies with chicks may provide valuable guiding information concerning the nutritive adequacy of combat rations.

The experiments presented in this report were conducted to determine which nutritional elements needed to be added to lyophilized samples of Individual Combat (C) and 5-in-1 Army rations in order to support satisfactory growth in chicks.

Experimental

Representative samples of the rations under investigation were frozen, and dehydrated in the frozen state under vacuum in a Desivac. Following dehydration, all the materials were ground in a food mill and thoroughly mixed. Analyses were conducted on the mixture to determine the levels of protein, fat, calcium, phosphorus, and most of the vitamins of the B complex. These analyses showed that the rations must be supplemented with calcium and phosphorus in order to meet the requirements of the chick for these mineral elements. Comparison of the protein : energy ratio showed that additional protein was also needed in order to meet the requirements of the chick for this nutrient. The composition of the basal diets used in the experiment is given in table 1. It contained 83.5 percent of lyophilized composite Army ration and 11.5 percent of purified casein which increased the protein content of the diet to approximately 25 percent. The diet was supplemented with methionine, since analyses indicated that the composite Army ration was deficient in this amino acid for the chick. The diet was also supplemented with glycine and arginine, two amino acids which are required specifically by the chick. The other ingredients included in the diet were based upon known nutritive requirements of the chick.

TABLE 1

Basal Army Ration Diet

Ingredient	Amount	Ingredient	Amount
	grams		*milligram*
Army ration [1]	83. 5	∝-Tocopheryl acetate	0. 50
Purified casein	11. 5	Menadione	0. 04
Methionine	0. 37	Choline chloride	0. 154
Glycine	0. 68		
Arginine	0. 16		I. U.
Dicalcium phosphate	3. 00	Vitamin A	450. 00
Pulverized limestone	0. 50	Vitamin D	150. 00
Manganese sulfate	0. 015		

[1] Composite Army ration, approximately 10 percent moisture, prepared by lyophilization.

Rhode Island Red × Barred Plymouth Rock male chicks were used in the experiments. Each lot contained 20 chicks at the start. They were identified with numbered wing bands at 1 day of age, weighed individually, and then placed on experiment. They were weighed individually weekly thereafter. The duration of the experimental period was approximately 4 weeks. The chicks were housed in electrically heated battery brooders equipped with wire mesh floors to prevent coprophagy. Feed and water were supplied *ad libitum*. A record of feed consumption was made at the time of each weighing. Observations for mortality were made daily.

A number of studies were conducted with the basal Army ration diet supplemented with calcium, phosphorus, and protein as indicated in table 1 to determine qualitatively and quantitatively the extent of B-vitamin deficiencies in these rations when used as the sole source of food for chicks. Additional studies were conducted to determine whether or not these rations are deficient in unidentified vitamins and trace minerals required by chicks.

Results

Qualitative studies.—Preliminary experiments showed that the composite Army ration is deficient in known B vitamins for chicks. A mixture of all of the known B vitamins produced normal growth. Pyridoxine, pantothenic acid, and folic acid appeared to be deficient, but the addition of these 3 vitamins alone did not support normal growth, indicating that at least 1 other B vitamin is also lacking in sufficient amounts for normal chick growth.

Experiment 1.—The first experiment was conducted with the basal C ration diet. This diet was fed alone and supplemented with all of the known B vitamins. Other supplements to the basal diet included pyridoxine, pantothenic acid, and folic acid, alone and in combination. The results of the experiment, presented in table 2, show that whereas the complete mixture of B vitamins promoted top growth, subnormal growth was obtained when pyridoxine, pantothenic acid, and folic acid were the only supplements to the basal diet. These results demonstrated that the basal diet is deficient in at least one other vitamin of the B complex.

TABLE 2

EFFECT OF SUPPLEMENTING THE ARMY C RATION DIET WITH CERTAIN B VITAMINS

Treatment	Average weight [1] 28 days	Feed per gram gain
	Grams	*Grams*
Basal C Ration	203(16)	1. 92
Basal C Ration + B vitamins [2]	367(20)	1. 85
Basal C Ration + pyridoxine HCl	203(17)	2. 05
Basal C Ration + Ca pantothenate	219(14)	1. 81
Basal C Ration + folic acid	198(18)	2. 17
Basal C Ration + pyridoxine HCl + Ca pantothenate	258(19)	1. 78
Basal C Ration + pyridoxine HCl + folic acid	196(18)	1. 99
Basal C Ration + Ca pantothenate + folic acid	221(18)	1. 95
Basal C Ration + pyridoxine HCl + Ca pantothenate + folic acid	246(18)	1. 78

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[1] Values in parentheses indicate number of chicks surviving at end of experiment. Each lot contained 20 RIR × BPR male chicks at start.

[2] The B vitamins were added to the basal ration to supply the following amounts in mg./100 gm: biotin, 0.02; Ca pantothenate, 2; pyridoxine HCl, 0.45; folic acid, 0.2; thiamine, 0.4; riboflavin, 1; niacin, 5; vitamin B_{12}, 0.0005.

Experiment 2.—In order to determine which of the other vitamins is deficient in the composite Army C Ration diet, a second experiment was conducted in which lots fed experimental diets lacking one of the B vitamins present in the B-vitamin mixture were compared with a lot given the basal ration plus the complete B-vitamin mixture and a lot fed the basal ration without vitamin addition. The plan of the experiment and the results are presented in table 3.

In this experiment, as in the previous one, the chicks fed the basal ration grew only at approximately one-half the rate of the lot fed the basal ration supplemented with B vitamins. Although the lot fed the basal ration with the vitamins minus biotin, attained a weight approximately 25 percent greater than that of the chicks fed the basal ration alone, the growth was significantly lower than that of the lot of chicks fed the basal ration plus the complete B-vitamin mixture. Omission of pantothenic acid and pyridoxine also caused some reduction in growth, but not to the same extent as when biotin was omitted. Omission of any of the other B vitamins apparently did not significantly affect the growth rate. No significant amount of mortality occurred in any of the lots except the one receiving the basal ration, where 50 percent mortality occurred.

TABLE 3

EFFECT OF SUPPLEMENTING THE ARMY C RATION DIET WITH VARIOUS COMBINATIONS OF B VITAMINS

Treatment	Average weight [1] 28 days	Feed per gram gain
	grams	*grams*
Basal C Ration	202(10)	2. 18
Basal C ration + B vitamins (B)	363(18)	1. 91
B—biotin	253(19)	1. 84
B—Ca pantothenate	342(18)	1. 80
B—pyridoxine HCl	345(19)	1. 81
B—folic acid	361(19)	1. 73
B—thiamine	361(18)	1. 75
B—riboflavin	377(18)	1. 84
B—niacin	378(19)	1. 60
B—vitamin B_{12}	379(19)	1. 81

[1] Values in parentheses indicate number of chicks surviving at end of experiment. Each lot contained 20 RIR × BPR male chicks at start.

The results of this experiment showed that the basal Army C Ration diet is deficient in biotin as well as pyridoxine and pantothenic acid for chick growth. A repetition of this experiment confirmed these results.

Experiment 3.—In an effort to determine the separate effects of biotin, pantothenic acid, and pyridoxine, the basal diet was fed together with all of the B vitamins and then with various vitamin mixtures in which one or more of the three under investigation were omitted singly or in combination. The results of the experiment are presented in table 4. In each case the most marked depression in growth occurred whenever biotin was omitted from the mixture. However, the omission of both pyridoxine and pantothenic acid caused an appreciable reduction in growth even when biotin was added to the diet. The results of this experiment show that the lyophilized composite Army C ration is definitely deficient in these three vitamins for chick growth.

Quantitative studies.—According to the results presented above, the Army C ration is deficient in pantothenic acid, pyridoxine, and biotin for chick growth. Further studies were conducted, therefore, to determine the quantitative extent of these deficiencies.

TABLE 4

EFFECT OF SUPPLEMENTING THE ARMY C RATION DIET WITH VARIOUS COMBINATIONS OF B VITAMINS

Treatment	Average weight,[1] 32 days	Feed per gram gain
	Grams	*Gram*
Basal C ration	183. 3 (18)	2. 60
Basal C ration +B vitamins (B)	408. 0 (20)	1. 82
B—biotin, Ca pantothenate, pyridoxine	174. 3 (16)	2. 46
B—biotin, pyridoxine	210. 4 (15)	2. 49
B—biotin, Ca pantothenate	221. 4 (18)	2. 32
B—pyridoxine, Ca pantothenate	271. 0 (17)	2. 13
B—biotin	253. 0 (18)	2. 32
B—Ca pantothenate	324. 2 (17)	1. 93
B—pyridoxine	333. 8 (17)	2. 04

[1] Values in parentheses indicate number of chicks surviving at end of experiment. Each lot contained 20 RIR × BPR male chicks at start.

The basal Army ration diet was the same as those used in the previous investigations. All of the B vitamins were added to the basal diet except for the one under investigation. At the same time similar groups of chicks were fed a purified diet containing all nutrients except the vitamins under investigation. The composition of the purified diet is presented in table 5. When this diet was used to determine the growth response curve for 1 of the vitamins the other 2 vitamins were added in adequate amounts to the basal diet.

TABLE 5

COMPOSITION OF PURIFIED DIET

Ingredients	Percent	Ingredients	Percent
Purified casein	18	Vitamin A supplement (10,000 A/gm.)	0. 08
Gelatin	5	D-activated animal sterol (1,500 D/gm.)	. 03
Fish meal, menhaden, 60 percent protein	4	Dicalcium phosphate	1. 9
Hydrogenated cottonseed oil	2	Limestone, ground	1. 2
Cellulose, pulverized	3	Dipotassium phosphate	. 5
Corn starch	62. 5	Sodium chloride	. 5
1-Arginine HC1	. 4	Magnesium sulfate	. 25
dl-Methionine	. 3	Trace minerals and vitamins[1]	. 4

[1]

	gm./100 kg.		*gm./100 kg.*
$FeSO_4.7H_2O$	54. 00	i-Inositol	100. 00
$MnSO_4$	32. 00	Niacin	5. 00
KI	3. 00	α-Tocopheryl acetate	4. 00
$CuSO_4.5H_2O$	1. 45	Menadione	1. 00
$ZnCl_2$	1. 00	Riboflavin	1. 00
$CoCl_2.6H_2O$	. 20	Thiamine chloride	1. 00
$Na_2MoO_4.2H_2O$	5. 00	Folic acid	. 100
Choline chloride	200. 00	Vitamin B_{12}	. 001

Purified diet.—The growth responses obtained upon supplementing the purified diet with graded levels of biotin are presented in table 6. According to microbiological analysis of the ingredients used in the purified diet, the basal purified diet contained 2 μg. biotin per 100 gm. of diet. Maximum growth was obtained when the diet was supplemented with 10 μg. of biotin, thus making a total of 12 μg. of biotin per 100 gm. of diet as the biotin requirement of the chick.

TABLE 6

GROWTH RESPONSES TO GRADED LEVELS OF BIOTIN

(Purified Diet)

Treatment	Average weight, 32 days
	Grams
Basal diet (no added biotin)	211
Biotin, 2.5 μg./100 gm	281
Biotin, 5 μg./100 gm	331
Biotin, 10 μg./100 gm	378

The results presented in table 7 show the growth responses when the basal purified diet was supplemented with pyridoxine hydrochloride. Since the microbiological analysis of the ingredients used in the purified diet showed this diet to contain 10 μg. of pyridoxine hydrochloride per 100 gm. of diet, the levels indicated in the table must be increased by this amount. Maximum growth was obtained with the highest levels of pyridoxine supplementation which corresponded to a total pyridoxine level of 410 μg. per 100 gm. of diet.

TABLE 7

GROWTH RESPONSES TO GRADED LEVELS OF PYRIDOXINE HYDROCHLORIDE

(Purified Diet)

Treatment	Average weight, 32 days
	Grams
Basal diet (no added pyridoxine)	83
Pyridoxine.HCl, 100 μg./100 gm	270
Pyridoxine.HCl, 200 μg./100 gm	358
Pyridoxine.HCl, 400 μg./100 gm	397

The growth responses to pantothenic acid additions are presented in table 8. According to the microbiological assays, the basal diet contained 50 μg. of calcium pantothenate per 100 gm. of diet. It is apparent that the highest level of calcium pantothenate, 850 μg. per

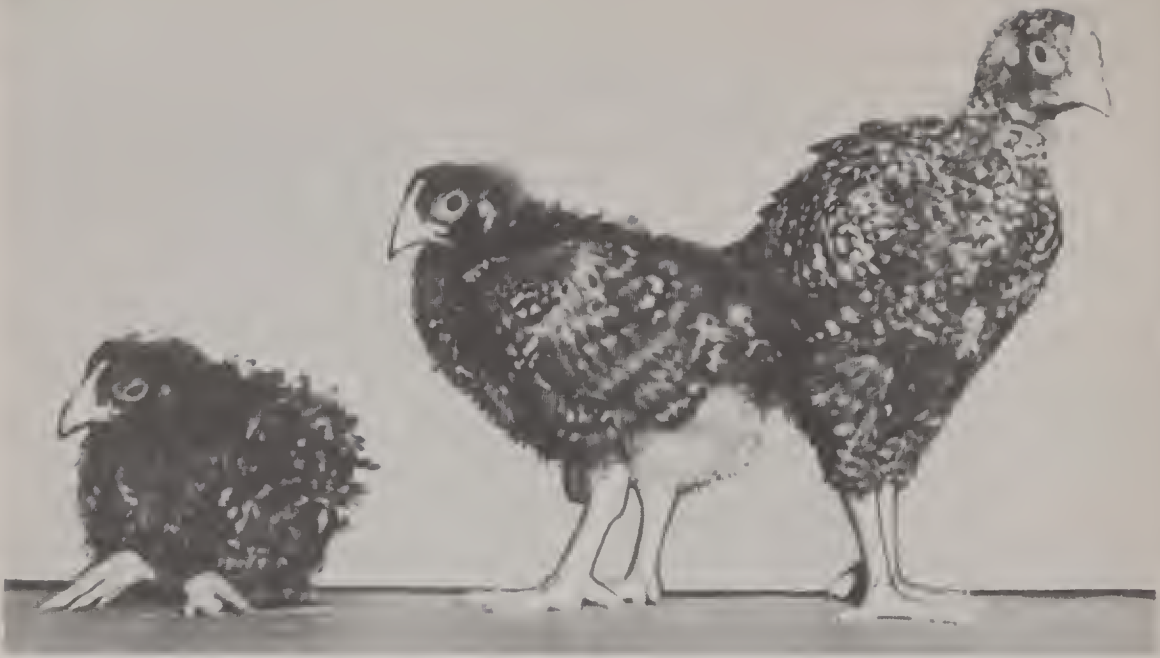


FIGURE 1.—Biotin response in chicks receiving supplemented C rations.

100 gm. of diet, did not promote maximum growth, showing that the pantothenic acid requirement of the chicks was somewhat higher than this amount.

TABLE 8

GROWTH RESPONSES TO GRADED LEVELS OF CALCIUM PANTOTHENATE

(Purified Diet)

Treatment	Average weight, 32 days
	grams
Basal diet (no added pantothenic acid)	96
Calcium pantothenate, 200 μg./100 gm	166
Calcium pantothenate, 400 μg./100 gm	307
Calcium pantothenate, 800 μg./100 gm	355

C ration.—The growth responses resulting from the addition of biotin to the Army C ration basal diet are presented in table 9. Growth increased in direct proportion to the increased levels of biotin added to this diet. The highest level added, 4 μg. per 100 gm. of diet, failed to produce top growth showing that somewhat more than 4 μg. per 100 gm. of diet must be added to this diet in order to make it complete for chicks. Figure 1 shows representative chicks receiving the basal C ration diet without biotin and with 2 μg. and 4 μg. added biotin per 100 gm. of diet.

TABLE 9

GROWTH RESPONSES TO GRADED LEVELS OF BIOTIN

(Army C Ration Diet)

Treatment	Average weight 32 days gm.	Efficiency gm. feed/ gm. gain	Mortality percent (after 1 week)
Basal C Ration (no added biotin)	256	2. 03	25
Biotin, 1 µg./100 gm	307	1. 93	15
Biotin, 2 µg./100 gm	338	1. 80	10
Biotin, 4 µg./100 gm	359	1. 69	5

The results presented in table 10 show the effects of additions of pyridoxine to the Army C ration basal diet. These results indicate that the diet is only slightly deficient in pyridoxine, since the addition of 50 µg. of pyridoxine hydrochloride per 100 gm. of diet increased the growth to near maximum levels.

TABLE 10

GROWTH RESPONSES TO GRADED LEVELS OF PYRIDOXINE HYDROCHLORIDE

(Army C Ration Diet)

	Average weight 32 days gm.	Efficiency gm. feed/ gm. grain	Mortality percent (after 1 week)
Basal C Ration (no added pyridoxine)	362	1. 81	5
Pyridoxine. HCl, 50 µg./100 gm	392	1. 63	------------
Pyridoxine. HCl, 100 µg./100 gm	411	1. 65	------------
Pyridoxine. HCl, 200 µg./100 gm	438	1. 55	5

The curve resulting from supplementing the Army C ration diet with calcium pantothenate is presented in table 11. The graded response to the addition of pantothenate shows the extent of the deficiency of this ration in terms of pantothenic acid.

TABLE 11

GROWTH RESPONSES TO GRADED LEVELS OF CALCIUM PANTOTHENATE

(Army C Ration Diet)

Treatment	Average weight, 32 days, gm.	Efficiency gm. feed/ gm. gain	Mortality percent (after 1 week)
Basal C ration (no added pantothenic acid)	250	2. 11	35
Calcium pantothenate, 50 µg./100 gm	272	1. 94	25
Calcium pantothenate, 100 µg./100 gm	358	1. 75	5
Calcium pantothenate, 200 µg./100 gm	377	1. 65	------------

Data are also presented in tables 9, 10, and 11 showing the improvement in the efficiency of feed utilization which resulted upon supplementing the basal C ration diet with the vitamins under investigation. The figures for mortality show that both the biotin deficiency and the pantothenic acid deficiency were severe enough to produce appreciable mortality, which was decreased as these vitamins were added to the diet.

5-in-1 ration.—The results presented in tables 12, 13, and 14 show the effects of adding these vitamins to the 5-in-1 basal ration diet. The results with the 5-in-1 ration agree in general with those obtained using the C ration diet, except that the 5-in-1 ration diet was slightly more deficient in biotin and pyridoxine than the C ration diet.

TABLE 12

GROWTH RESPONSES TO GRADED LEVELS OF BIOTIN

(Army 5-in-1 Ration Diet)

Treatment	Average weight, 32 days
	grams
Basal 5-in-1 (no added biotin)	244
Biotin, 1 μg./100 gm	254
Biotin, 2 μg./100 gm	303

TABLE 13

GROWTH RESPONSES TO GRADED LEVELS OF PYRIDOXINE HYDROCHLORIDE

(Army 5-in-1 Ration Diet)

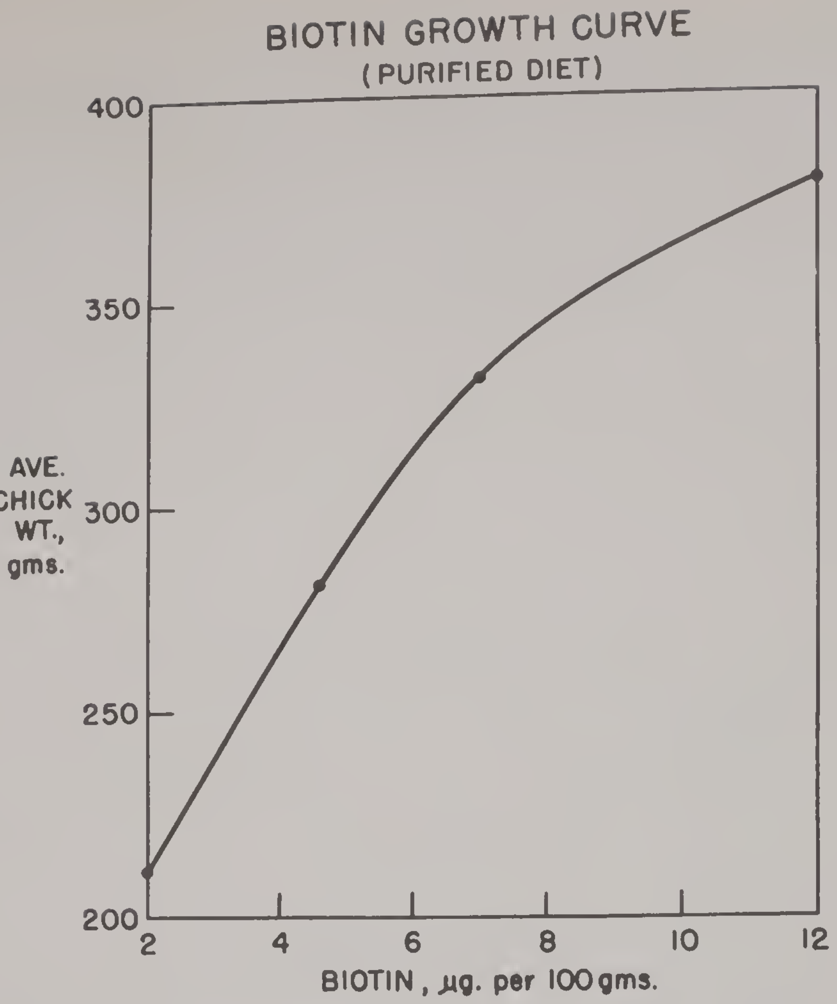
Treatment	Average weight 32 days
	grams
Basal 5-in-1 Ration (no added pyridoxine)	309
Pyridoxine. HCl, 50 μg./100 gm	344
Pyridoxine. HCl, 200 μg./100 gm	353

TABLE 14

GROWTH RESPONSES TO GRADED LEVELS OF CALCIUM PANTOTHENATE

(Army 5-in-1 Ration Diet)

Treatment	Average weight, 32 days
	Grams
Basal 5-in-1 (no added pantothenate)	270
Calcium pantothenate, 100μg./100 gm	306
Calcium pantothenate, 200 μg./100 gm	338
Calcium pantothenate, 400 μg./100 gm	372



FIGURS 2.—Biotin response in chicks receiving the purified diet.

The results obtained using the purified diet were plotted on graph paper and used as standard curves for calculation of the amounts of the various vitamins present in the Army ration diets. A representative curve, showing the response to biotin is presented in figure 2. These values were then compared to those obtained by microbiological analysis. The results for the Army C ration diet are presented in table 15, and those obtained with the 5-in-1 ration diet are presented in table 16.

TABLE 15

BIOTIN, PYRIDOXINE, AND PANTOTHENIC ACID CONTENT OF ARMY C RATION DIET

Vitamin	Amount per 100 grams of diet	
	Chick assay	Microbiological assay
	µg.	µg.
Biotin	4. 5	4. 0
Pyridoxine	210	176
Pantothenic acid	600	629

TABLE 16

BIOTIN, PYRIDOXINE, AND PANTOTHENIC ACID CONTENT OF ARMY 5-IN-1 RATION DIET

Vitamin	Amount per 100 grams of diet	
	Chick assay	Microbiological assay
	µg.	µg.
Biotin	3. 1	
Pyridoxine	140	121
Pantothenic acid	575	600

Sparing effect of folic acid upon biotin requirement.—In earlier studies, indications were obtained that the basal C ration diet might be deficient in folic acid. An experiment was conducted, therefore, in which the C ration diet was fed with all B-vitamin supplements except for biotin and folic acid and then with levels of folic acid in the presence and absence of added biotin.

The results of the experiment, presented in table 17, show that the addition of folic acid in the absence of added biotin caused a marked, but sub-optimal increase in growth. The addition of biotin in the absence of folic acid caused maximum growth which was not improved by the further addition of folic acid.

TABLE 17

EFFECT OF SUPPLEMENTATION OF ARMY C RATION DIET WITH FOLIC ACID IN PRESENCE AND ABSENCE OF ADDED BIOTIN

Treatment	Results at 31 days		
	Average weight gm.	Efficiency gm. feed/gm. gain	Mortality percent
Basal C Ration diet (no added biotin or folic acid)	180	2. 43	40
Folic acid, 10 µg./100 gm	226	1. 98	35
Biotin, 20 µg./100 gm	305	1. 78	20
Biotin, 20 µg./100 gm + Folic acid, 10 µg./100 gm	308	1. 78	1515

Unknown vitamins and trace minerals.—A number of experiments were conducted in which the growth of chicks receiving the basal Army C ration diet plus the complete B-vitamin mixture was compared with the growth of similar lots of chicks receiving further supplements of 4.2 percent desiccated, defatted whole liver and 2 percent fish solubles as sources of unknown chick growth factors and fu supplements of trace minerals. Representative results of these stu are shown in table 18. In another experiment it was found that su plementing the basal diet plus added B vitamins with 5 percent dried brewer's yeast caused no improvement in chick growth. The small increase in growth resulting from the addition of liver and fish solubles may be due to a slight deficiency in the Army ration diet in terms of unknown vitamins required by chicks. However, this deficiency appears to be relatively insignificant in comparison to the deficiency of the three known vitamins.

TABLE 18

EFFECT OF SUPPLEMENTING ARMY C RATION DIET WITH TRACE ELEMENTS AND SOURCES OF UNKNOWN VITAMINS

Treatment	Average weight 32 days	Feed per gm. gain
	grams	*grams*
Basal C Ration diet + B vitamines (B) [1]	[1] 408(20)	1. 82
B + trace elements [2]	398(19)	1. 85
B + 4.2 percent, dried, defatted liver + 2 percent fish solubles	428(19)	1. 86
B + trace elements: + 4.2 percent dried, defatted liver + 2 percent fish solubles	445(20)	1. 80

[1] Values in parentheses indicate number of chicks surviving at end of experiment. Each lot contained 20 RIR × BPR male chicks at start.

[2] Minerals were added to the basal ration to supply the following amounts in mg./100 gm.: Fe, 10; I, 2; Cu, 0.38; Zn, 0.48; Co. 0.049.

The nutritive value of Army combat rations has been studied previously by Sporn and Elvehjem (*2*) using rats and by Sporn, Ruegamer, and Elvehjem (*3*) using monkeys. In each case, the Army rations required special supplementation with vitamins in order to produce satisfactory growth in the experimental animals.

It is evident from the results presented in this report that the Army rations are seriously deficient in biotin and pantothenic acid, and moderately deficient in pyridoxine, and slightly deficient in unknown vitamins for chick growth.

Under the conditions of these experiments, folic acid appeared to have a sparing effect upon the requirement for biotin. Whether this sparing effect occurred within the animal body metabolism or indirectly through stimulation of intestinal microbiological synthesis of biotin cannot be determined from these experiments. The results do explain the earlier indications that Army rations might be deficient in folic acid, and show that this is not the case when the diet is supplemented with adequate biotin.

The chick is known to have higher requirements than many other species for most of the vitamins. It is this fact that makes the chick an excellent experimental animal for use in biological assays for vitamins. On the other hand, the results of these studies with chicks cannot be construed to indicate that Army rations are necessarily deficient in biotin, pantothenic acid, and pyridoxine for man. Since packaged rations, for the most part, consist of canned foods, these results may indicate the desirability of investigating the processing loss of these vitamins in home- and commercially-canned foods in general use in this country. Studies of this nature are very limited (*1*).

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Discussion

CHAIRMAN GRIFFITH:

Is there any evidence that this apparent increased requirement for pyridoxine could in any way be due to a possible pyridoxine antagonist in these rations?

ELVEHJEM:

I won't go into detail. I think we can answer that by saying that in the early work on the K Rations we did study that possibility and we were unable to demonstrate any clear evidence for antagonists in these rations. That does not mean they may not be present, but we were unable to clearly show such an effect.

A. J. CARLSON (University of Chicago):

I would like to bring this point out to all four of you men who have spoken this morning. You are using the term "growth," and that term is used, I understand, to mean body weight. That is a confusion. You will agree it is a confusing affair, because it may not be growth entirely, it may be obesity or fat.

Is there any other way of checking that? We tried a few years ago on rats to check it by bone length. That may not be accurate enough, but it seemed important. At any rate, I don't think we should put into the scientific literature that you are dealing with growth alone when you are measuring weight increase or decrease.

I think more work on animals in all species is called for on this Army ration problem. Would it be possible to add another check in the rat studies, namely, the life span? Certainly, that is only 2 or 3 years, and that is not very far in the interval between our wars.

CRAMPTON:

In Dr. Spector's comments, he said something about increased protein not being effective in the case of some of the rations tested with rats. I only want to comment that it might be found that increased protein would be effective if increased fat were simultaneously put in. We have found in working with fat that there are optimum levels of fat, not for fat *per se*, but there is a percentage to be an optimum ratio between the calories coming from protein and the calories coming from nonprotein sources. Consequently, when you add fat to a ration that is optimum, as we usually think of it in terms of protein, one of the first responses is, if the calories from protein have dropped below 17 or 18 percent of the total calories, there is appetite failure and this can be recovered by immediately bypassing it to give this ratio or something above it.

It seems then that the question of protein level, adequate or optimum protein level, for the full utilization of the total caloric value of the diet may not be a constant, and if we alter the concentration of calories per unit weight of diet, we may have to increase the protein concentration in question. I think there is evidence that the concentration of most of the nutrients including protein, perhaps, has some rather definite ratio to the concentration of energy per gram of diet.

HORWITT:

I would like to make a general comment about the use of rat growth data in evaluating human nutritional requirements. Over the past 12 years or so, this problem has been with us because all of the human diets used on the Elgin studies have been simultaneously tested with rats. However, we did find out during the course of this work that protein supplementation was almost always necessary in order to make the apparently adequate given diet adequate for the growing rat.

If we took the presently known National Research Council diet with 16 gm. of protein, you would have approximately 10 gm. of protein present in your diet. Assuming a weight of 600 gm., dry weight of diet, and that diet in its best form might very easily be inadequate for the rat. One would be tempted when one is not well aware of the pitfalls of this kind of work, to conclude that that diet is therefore inadequate.

POLLACK:

I think one of the points that Dr. Horwitt brought up is clear. You take the NRC standard, the result of one gram per kilogram for the growing rat, when the NRC standard for growing children is three grams per kilogram, and it is a question of applying it to the young. If you take the adult standards and give it to the growing rat, you get into a confusion of standards.

GEORGE:

I would like to ask Dr. Elvehjem a question, whether the 5-in-1 ration has been fed to these animals separately without any previous processing, i. e., in fresh form, and a higher requirement of B_6 found?

ELVEHJEM:

Do you mean the individual ingredients from the Army rations? No, it has not been done, but it should be done, perhaps. They ought to be processed in individual meals, processed according to the directions on the package and perhaps then you would get quite a different response in your monkeys than you do with everything mixed togther.

GLADYS EMERSON (Merck Institute of Therapeutic Research):

I would like to mention some work we have in progress on the apparent high requirement of the monkey for vitamin B_6, which is in agreement with Dr.

Elvehjem's findings. We started rhesus monkeys weighing 2.2 kg. on the best stock diet we knew, consisting of dog pellets, whole wheat grit, oranges, apples, cabbage, peanuts, and other vegetables. We kept the animals on this ration for a period of about 3 months, during which time they initially gained weight and then seemed to reach a plateau. We transferred them to a purified diet with 3½ mg. of B_6 given twice weekly, which may not be the equivalent of 1 milligram a day. But, the rate of growth increased tremendously during that period, and after the 5 weeks, in half of the monkeys, namely 6, we withdrew the B_6, and in the second weighing after 2 weeks, the animals started to lose weight and it was progressive. There was a dryness of the fur, a coarseness and a developing alopecia, and I agree with Dr. Carlson in regard to increase in weight as a standard of growth. That may not apply in all cases.

In fact, we have rats in which it does not apply, where we have thousand-gram rats that are merely obese. However, these animals that are deprived of B_6 are definitely emaciated. They are listless and they are extremely shy. It appears that the monkey has an exceptionally high requirement for vitamin B_6. It must be out of the range of the requirement for the human because the requirement of one milligram per day apparently is not in excess.

VI. Evaluation of Nutritional Status of Populations

CHAIRMAN GRIFFITH:

We have had the discussion of the evaluation of rations using procedures involving three of the lower species. There are those, I am sure, who would say about such experiments that they are interesting and fine, but what do they mean in terms of man? The ultimate, of course, is the actual evidence as found in the human.

Nutrition Survey Methods

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and

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It has been demonstrated repeatedly that the nutritional status of populations can be assessed with accuracy sufficient to be used as practical guides for feeding programs. The scientific literature contains numerous well-known examples of which the Newfoundland surveys might be cited as typical (*1*, *3*, *13*, *23*).

The same basic methods as used in the Newfoundland studies can be and have been applied under conditions of war or emergency but must be tailored to fit the specific situation according to limitations of time, personnel, and facilities, special characteristics of the population group, as well as the factors peculiar to a population under stress. The discussion to follow will focus on these special problems.

It must be emphasized that the nutritional status of a population cannot be established by the casual observations of nutritionally inexperienced persons. The fact that a cheering throng stands along the street as the liberators march in is no proof that the nutrition of the entire populace is adequate. Nor can one assess nutritional status by what one can observe in driving along the streets. This mistake has been made on several occasions. The assessment of nutritional status requires, in most instances, a careful and detailed examination of many people.

The methods available fall into five categories: Vital statistics, food intake, anthropometric, clinical examinations, and laboratory tests. In accordance with the program arrangement this presentation will omit consideration of clinical examination and laboratory tests. No one of the above methods by itself can be expected to yield a precise and always reliable measure of nutritional status. When used together, and carefully evaluated, they do provide a useful and rather accurate picture of a nutritional situation.

Vital Statistics

Such information can be used to supply *indirect* or *presumptive* evidence of nutritional status even though under war conditions this type of information is often unreliable. These statistics must be

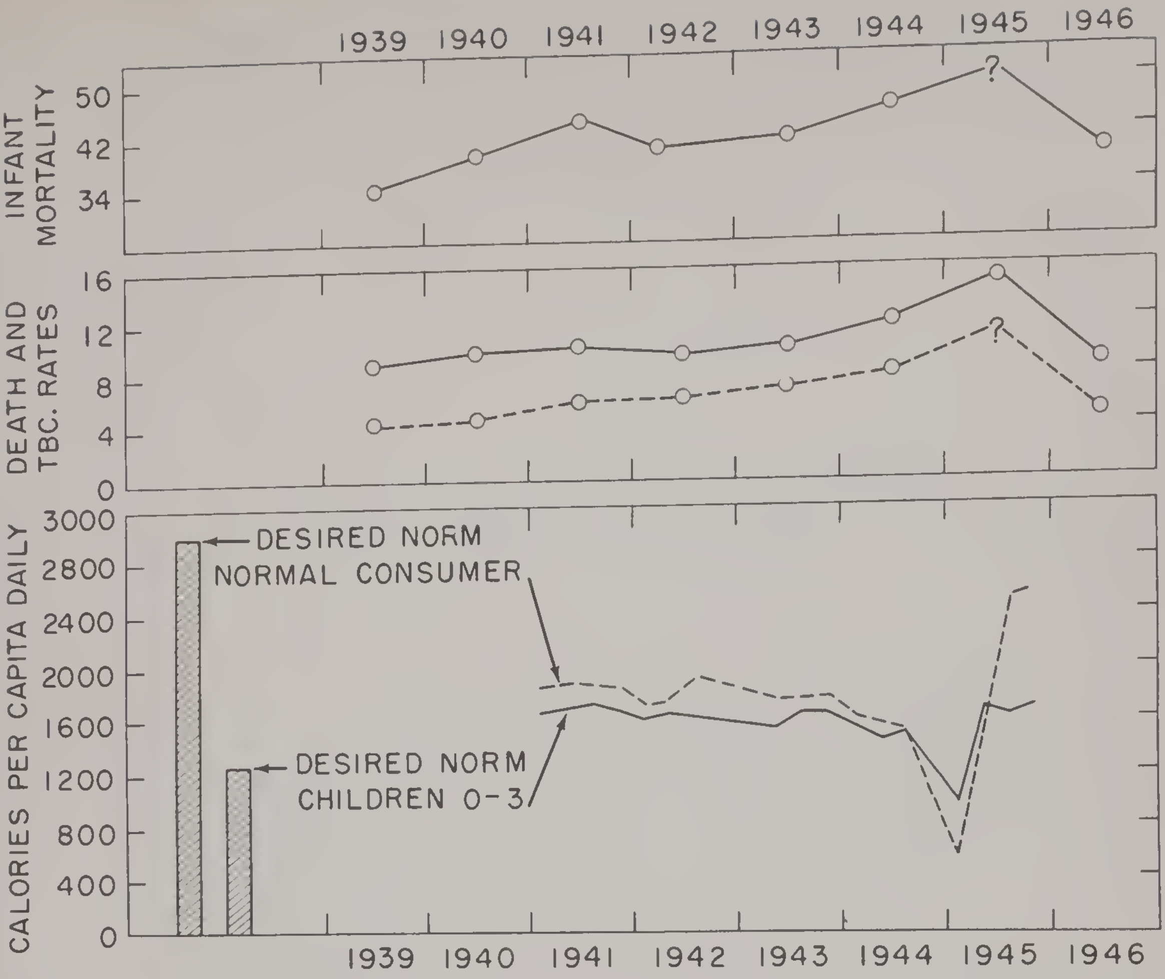

FIGURE 1. **Calorie intake in relation to certain vital statistics, the Netherlands, 1939–46.**

evaluated with great care and must be checked with more direct methods to be mentioned later. It is well known that each of these health indices is influenced by many environmental factors other than nutrition.

The best established of these indirect nutrition indicators are:

Crude death rate.—An otherwise unexplainable increase in this statistic constitutes presumptive evidence of malnutrition, especially if the increase is marked among the elderly. Figure 1 shows the crude death rate in the Netherlands in 1939–46 plotted with changes in calorie intake and other health indices (*7*). It will be noted that there was a small increase during the early years of the German occupation when the calorie intake was low, but close to long-term minimum maintenance levels. However, later, in 1944 and especially in the first 4 or 5 months of 1945, the food situation deteriorated markedly. It will be noted that the crude death rate increased about 100 percent. When death rates were calculated for large cities in the western Netherlands, which were afflicted the worst, increased mortality up to 200 percent was found as shown in table 1 (*7b*). Perhaps half of these excess deaths were due to outright malnutrition and half to other causes. Valaoras reported even more striking increases in deaths in Athens and Piraeus in 1941–42 (*35*), although this could not be accurately expressed as an absolute rate increase due to breakdown of normal vital statistics reporting and population shifts, a difficulty frequently encountered in time of war.

TABLE 1

MORTALITY IN CERTAIN LARGE CITIES IN WESTERN HOLLAND

City	Population	Number of deaths in first 6 months of—			Mortality relative to 1939—		
		1939	1944	1945	1939	1944	1945
Amsterdam	800, 000	3, 655	4, 393	9, 735	100	120	266
Rotterdam	640, 000	2, 616	3, 260	7, 827	100	125	299
The Hague	520, 000	2, 419	2, 940	6, 458	100	121	267
Utrecht	170, 000	776	1, 112	2, 065	100	143	266

Infant mortality rate.—This statistic reflects the effects of malnutrition on both mother and child. The degree to which the rate will increase depends not only on the severity of malnutrition generally, but on the degree to which expectant mothers and infants receive preferential treatment in the distribution of available food. Figure 1 reflects the correlation of infant mortality to other indicators and also depicts the marked preferential treatment of infants in the distribution of food. It is not surprising that only rarely could cases of specific nutritional deficiency be found in young children in western Holland (*7c, 33*) ; this was also true in Germany (*27*) and Austria (*9*).

Tuberculosis death rate.—An increased mortality from tuberculosis is a well-known companion to famine. This, too, is depicted in figure 1. As with the crude death rates, the increases in tuberculosis mortality were far greater in the large municipalities of western Holland than the change for the entire country which is plotted in figure 1. An unexpected and paradoxical finding in the Dutch studies was that in spite of the increased tuberculosis mortality, and an increased number of open cases who were forced to work and to move about in search of food, the percentage of children who gave positive tuberculin skin reactions decreased strikingly as shown in table 2 (*7d*). The explanation of this phenomenon is not apparent.

TABLE 2

PIRQUET TESTS IN AMSTERDAM, ROTTERDAM, AND THE HAGUE

Year	Examined	Negative percent	Weak positive percent	Strong positive percent
1941	900	71	8	21
1942	889	75	8	17
1943	952	79	1	20
1944	729	83	2	15
1945	757	92	3	5

Another limitation in the use of tuberculosis statistics appeared in Germany during and after World War II. A special ration was provided for the tuberculous. When food became scarce the number of people recorded as tuberculous increased markedly (*30*).

Other statistics.—Maternal and stillbirth death rates do not seem to be as useful as the above statistics. It is now generally agreed that newborn length and weight is not a sensitive indicator although small effects may be found with careful study (*10*). Birth rates may show a rise or a fall during periods of food stress. So many factors affect this rate that it has little usefulness as a nutrition indicator.

The percentage of women showing menstrual irregularities, delayed menarche, or amenorrhea is useful if such figures are available and there are appropriate baselines. For example, Valaoras reported that 70 percent of the adult females in Athens and Piraeus became temporarily amenorrheic during the famine in Greece in 1941–42 (*35*). Japanese surveys in 1946–47 showed an incidence of 10 to 20 percent "delayed menstruation" (*16*). In 1950 with a generally improving food situation, these figures decreased to range from 8 to 10 percent. On the other hand, the percentage of women showing "deficient lactation" was little different in 1950 from that in 1946 (*2*).

The association of famine with a decrease in deaths from diabetes generally observed during World War I and II deserves further study as an indicator of nutrition (*20b*). It is well known that the incidence of peptic ulcer rises during the stress of war but it is doubtful that this is due primarily to undernutrition.

Analysis of police records to discover the location and frequency of food thefts may provide some indication of the severity of food shortages. The number of beggars on the streets, the number on relief rolls, and the amount of labor unrest may provide some indication of undernutrition. Where compulsory health insurance schemes are in effect, useful information may be gained by examining their medical records. However, this source of information is subject to error where certain medical diagnoses provide extra rations and where malingering to escape draft calls is encountered.

Additional Indirect Methods

Food supply data.—Useful information can be obtained if reliable data are available on total food production, imports and exports, and population composition. Such data can be used not only to evaluate the quantitative adequacy of the food but its qualitative adequacy as well. While it is quite true that European experience during World War II clearly indicated that calories were the all-important consideration, and that detailed consideration of nutrient intake was not informative or helpful (*11*), it should be noted that this was not a general experience in all theaters of war (*19, 24, 32*). Before the concept of the all-important calorie becomes established as a principle in future military planning, it should be noted that the validity of this concept is predicated on the availability of the types of foodstuffs available in the European Theater during World War II. This might be quite different in future situations.

Overall food supply data can be translated into per capita food and evaluated against a "yardstick" to be described later. A correction for 10 to 15 percent loss between food at retail and food consumed is ordinarily applied. This figure may be considerably less when food is very short. It must also be remembered that the significance of per capita food figures may be influenced by rationing schemes, transportation bottlenecks, food thefts, black-market operations, public feeding practices, disproportionate military requirements, and amounts diverted to animal feed or industrial uses.

A unique opportunity to utilize the total food supply principle arose during the Berlin Blockade since the Berliners were almost totally dependent on the airlift for food (*6*). In this instance Berry, Cowin, and Magee could determine with considerable precision that the per capita food intake was about 1,800 calories per day, a level which is borderline if appreciable work is expected from the population. Moreover, the rationing system in effect was unrealistic in that it failed to allow sufficient food for adolescents, especially boys, workers, and men of large stature. Evidence of malnutrition was especially noticeable among these groups. This situation also provided a unique opportunity to follow the response to ration increases as will be discussed later.

Rationing schemes.—An examination of official ration allowances for various groups in relation to their special needs often helps in forming preliminary estimates of food adequacy. Nearly always such data must be checked by dietary surveys. Consultation with local rationing officials was helpful in the rapid "spearhead" surveys made in western Holland and in Germany immediately following World War II (*12, 33*).

Local public health and medical practitioners.—Consultation with such officials is especially helpful in the spearhead type of survey referred to above and is essential in obtaining cooperation and assistance for any more detailed examinations to be done.

A unique method was applied by the British in following the nutritional status of the Channel Islanders when Jersey and Guernsey were occupied by German forces during World War II. Periodic aerial photographic surveys were made and from these the number of cattle in the fields estimated. The British reasoned that if starvation was developing the cattle would be slaughtered for food. In this manner they were able to reassure themselves that the Channel Islanders were being reasonably well fed. Subsequent experience bore out their thesis (*21*).

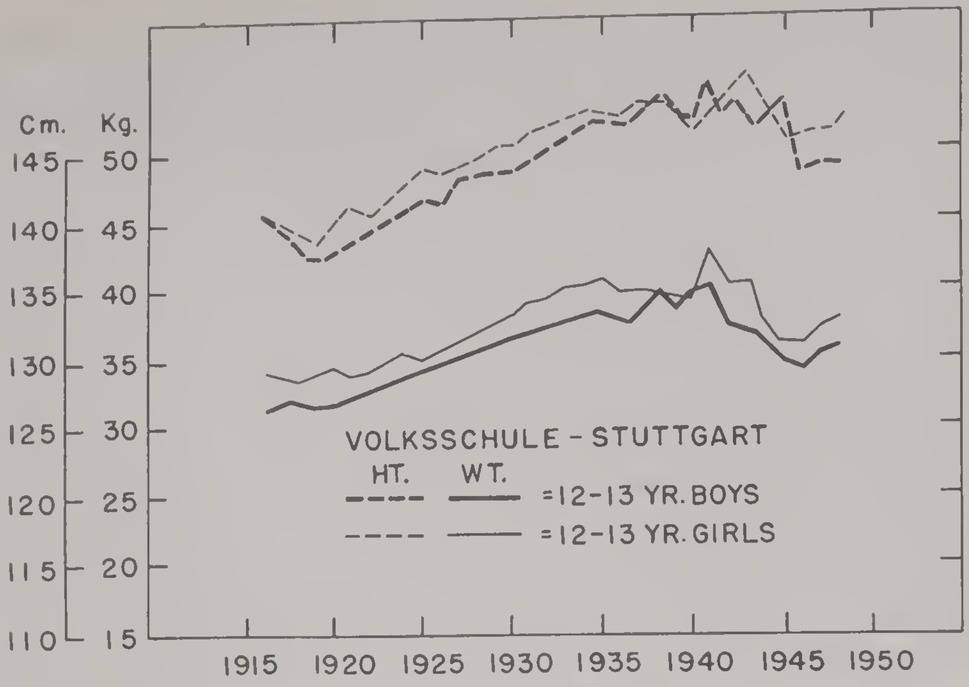

FIGURE 2.—*Growth of boys and girls aged 12 to 13 in Stuttgart, Germany, 1916–48.*

Direct Methods

Anthropometric.—Measurements of body weight and height as related to sex and age offer the most objective and probably the most sensitive direct measure of nutritional deterioration. Such measurements can be obtained quickly and expressed quantitatively. The significance of height-weight deviations can be evaluated against a base line standard, if a suitable one is available; alternatively, these measurements can be made periodically and changes correlated with food intake.

(*a*) *In children.*—There are several factors which must be kept in mind in evaluating the normalcy of growth in children. First, it is well known that there is a seasonal effect on growth, that is, children tend to gain in height but little in weight during the summer months, and vice versa, gain more in weight than in height during the winter (*20*, *26*). Secondly, in most occidental countries there has been a slow but relatively constant increase in growth rate so that children tend to be taller and heavier for a given age (*22*, *36*). Thus the fact that a given group of children have the same height and weight for age and sex as a similar group 20 or so years previously may conceal the fact that they were suffering from undernutrition in that food had not been sufficient to permit them to attain their expected height-weight status (*35*). This is brought out in figure 2. It will be noted that following the World War I period, there was a rather steady increase of height and weight up to the World War II period. During the latter period 12- to 13-year-old children not only failed to continue their expected gains in height and weight but actually showed a loss. These data are taken from the study of Howe and Shiller on school children in Stuttgart, Germany. Children of other ages showed similar changes (*17*). Thirdly, it is well known that there are substantial differences in average height and weight between children of low and upper economic groups—the latter being greater (*5, 14, 22*). Fourthly, it is known that many environmental factors other than nutrition affect the growth of children. However, the fact that inadequate nutrition was a primary factor in the restricted growth of children observed in Holland (*7c*), France (*20c*), and Germany (*17*, *20c*) during or after World War II is strengthened by the fact that children in England, where nutrition was maintained, did not show growth failure; rather they continued to grow more rapidly except possibly for short breaks in their growth curves during 1941 and during the severe winter of 1946–47 when food was slightly short (*8*, *36*). The Berlin Blockade also afforded a unique opportunity for Berry *et al.* (*6*) to relate nutrition to growth of children. Children examined initially in November 1948 early in the Blockade were considerably below standards of height and weight for London children. A ration increase of approximately 200 calories to yield per capita calories of about 2,000 daily (with some redistribution in rations) produced increases in height and weight by October 1949 so that these children were much closer to London standards, thus constituting good evidence that these children were undernourished at the time of the original examination.

There is some difference of opinion as to the age and sex of children most sensitive to nutritional deprivation. The British and most other observers agree that males of all ages usually show greater effects during food shortages (*4*) but this has not been a universal experience (*35*). In general, most European studies have found the 9- to 14-year age group to show the greatest effects. It is uncertain whether the sensitivity of this group is due to physiologic differences or to the usual rationing scheme, or both.

In general it can be stated that growth failure in children is an established and valuable index of nutrition. However, there are many pitfalls in using children for such purposes, most of which can be avoided if the samples are carefully selected and evaluated. Greenberg and Bryan have shown that the statistical technique of analysis of covariance markedly reduces the size of samples needed (*14*).

(*b*) *In adults.*—If one had to select only one from the many measures which have been used to assess undernutrition, serial body weights and heights of adults would unquestionably be the method of choice. Such weights can be obtained rapidly, with good accuracy, and respond quickly to changes in food intake. The results can be expressed as a ratio to normal standards for the particular population group. If such standards are not available the results can be related to arbitrarily selected standards. In this instance the results are far more reliable when serial determinations of the same population group show a consistent trend toward or away from the arbitrary standard.

Following World War II, it became necessary to follow the nutritional status of the German people over a prolonged period. The survey procedure which gradually evolved was based primarily on periodic mass weighings of adults and children, supplemented by detailed surveys of nutrition teams. The mass weighing program resulted in the collection of hundreds of thousands of observations obtained from unselected groups found in queues, factories, street corner stations, and schools. In this program, only weight, age, sex, and geographic location were recorded. The hundreds of thousands of observations made each month tended to cancel out sampling errors. Heights were not taken. Body weights were then analyzed to yield average weights for the various age-sex groups. Changes in these averages from month to month were used to estimate the need for changes in the official ration.

In addition, these data were expressed as a percent of "standard" weight for the various age-sex groups as shown in table 3 (*24*). It will be noted that men of each age group were rather consistently further below their standard than were women. The older age groups, both men and women, were definitely below their standard, which is in accord with the general observation that the older age groups are more severely afflicted when food is short. As shown in table 3, there was a general decline in body weights in the November–December period in spite of an increase of about 200 calories in the official ration. This was undoubtedly due to a decrease in extra-ration food. The lack of garden produce at this time of year, difficulties in foraging for food in winter months, a decrease in environmental temperature, and lack of adequate heating in homes could explain this phenomenon.

TABLE 3

Body Weight of Adults in Ten Largest Hessian Cities (Germany) in 1946

Official ration calories/day		1250–1350 May–July	1330–1340 August–October	1550 November–December
		Body weight—percent of standard		
Women	18–39	99. 0	102. 0	100. 0
	40–59	91. 5	94. 0	93. 0
	60+	90. 0	89. 0	88. 0
Men	18–39	97. 0	96. 5	95. 5
	40–59	93. 0	92. 0	87. 5
	60+	92. 0	91. 5	87. 0

The extensive data which accumulated from the mass weighing program in Germany in 1945–47 has never been critically analyzed to check its validity as a precise measure of changes in nutritional status. The method is open to error from inadequate sampling, inaccuracy of weighing scales, varying amounts of clothing, and inappropriate standards. If an appreciable amount of hunger edema is present, real weight losses may be obscured. The "normal" incidence of obesity in a population may also influence the results. However, many of those who were personally involved in this program feel that it had real value and validity as well as being a powerful psychological weapon in reassuring the people that their health was a matter for concern and action by the occupation authorities (*18*, *31*). Others, including the authors of this report, feel that more accurate results could have been obtained from the examination of much smaller numbers of people carefully selected to obtain a population cross section using a method similar to that used by Davidson *et al.* (*9*) in Vienna in which height as well as weight, age, and sex were recorded in a standard fashion. This permits comparison to standard height-weight tables and the sample can be divided into those whose weight for height (and sex) is in the normal range, and the percent which fall above or below. On subsequent resurveys of the same groups a shift to either the right or left will have significance. Height need not be recorded, even in small samples, if the same individuals can be resurveyed periodically. However, experience has shown that it is difficult to keep such groups intact. Heights are then needed to see whether the results are biased by the individuals who did not appear for reexamination.

The collection of height-weight data from both adults and children is ordinarily confined to the larger cities. It has been a general experience that rural populations, being food producers, maintain a far more satisfactory nutritional state than city groups since they must depend primarily on commercial food procurement which is strictly controlled.

Personnel who collect height-weight data must be carefully trained and instructed if reliable and uniform results are to be obtained. The scales must be checked for accuracy periodically. Accurate heights will not be obtained unless care is used in having the subject stand fully erect. A standard procedure with respect to amount of clothing and the presence or absence of shoes must be followed.

Dietary surveys.—Detailed information on food intake constitutes an essential check on other methods even though such studies can ordinarily be done on only a fraction of those who are measured, or given clinical examinations. Such surveys have their inaccuracies especially under condition of severe food shortage when people may be reluctant to reveal their exact food intake for a variety of obvious reasons. Special measures and skillful observers are needed.

The information may be collected in several ways:

(*a*) The 24-hour recall method is rapid and will provide good preliminary results if trained observers are available. It is helpful to use food check lists and models of typical portions of usual foods to increase accuracy.

(*b*) A 3-day record of food consumed may be kept by housewives for themselves and families. Careful instruction and checking of results are necessary.

(*c*) Observers may be placed in homes to weigh and record food intake.

(*d*) Institutions may be required to keep accurate records of all food procured or produced and served. Institutionalized groups are generally at the mercy of the official rationing procedure and are unable to forage for food. Thus, they may be especially vulnerable.

Selection of samples.—The sample to be surveyed will vary according to the prevalence, severity, and type of malnutrition, the purpose of the survey and many other factors. In general, three types of surveys have been done.

(*a*) Rapid spearhead surveys are useful for preliminary estimates of the general situation especially when malnutrition is marked. Under this procedure preliminary consultations are held with local rationing and health authorities and with leading medical practitioners to obtain preliminary information and to enlist their cooperation in obtaining the subjects desired for examination. No attempt is made to obtain a true cross section of the population. Rather, an attempt is made to select a small group of 150 to 200 subjects who would be expected to be most severely afflicted. A brief history, rapid clinical examination, and height-weight measurements are made on each. Ten percent of the group are used to obtain 24-hour diet histories. Local hospitals and institutions are visited and rapidly inspected.

(*b*) Detailed surveys follow the same preliminary pattern of consultation with local officials. However, in this instance an effort is made to obtain a more representative cross section of the population including all economic groups, both children and adults, laborers, and other special groups. Infants, pregnant and lactating females will generally receive special treatment and their nutritional situation will already be known. Care should be taken that the adult sample includes people over 50 years of age. Medical examination and heights and weights are taken on all of each sample, diet histories on 20 percent, and laboratory examinations on 25 to 50 percent (if facilities are available). The size of the sample required will obviously be influenced by the frequency and severity of the nutritional defects encountered. It is estimated that for a city of 100,000 population a total sample of about 2,500 would be required on the initial survey. On subsequent resurveys a properly selected sample of 600 would suffice for comparative purposes. As an illustration, if nutritional edema were found to occur in 1 per cent of the individuals during the original survey and at the time of a subsequent survey the prevalence was 3 percent or more, then the sample of 600 would be adequate to show a rise which is statistically significant. On the other hand, when the original survey shows a higher prevalence, for example 10 percent of subjects underweight, a sample of 600 would be more than adequate to detect a 100 percent increase in the defect. In this instance as few as 200 individuals would suffice (*28*). Davidson *et al.* in their studies in Vienna in 1945 surveyed approximately 1 per cent of the total individuals in certain food ration classifications, namely, heavy workers, workers, employees (white collar workers mainly), children under 14 and "others" (*9*).

(*c*) In addition to and following the detailed surveys the usual practice in Germany was to obtain large numbers of adult weights by random street weighings and weights of children by mass school weighings. The following guidelines were promulgated in November 1945 for the American Forces in Germany (*15*):

1. The following minimum number of persons will be weighed monthly in every community of 10,000 population and over, according to the size of the community:

10,000–50,000 population, 5 percent to be weighed.
50,000–100,000 population, 1½ percent to be weighed.
100,000–500,000 population, 0.75 percent to be weighed.
1 million and over population, 0.5 percent to be weighed.

2. Body weight (to the nearest 1 pound or 0.5 kg.) of male and female adults 20 through 39; 40 through 59; 60 and over.

3. Individuals should be selected at random from public places such as queues, street corners, etc.

4. Individuals should be weighed with shoes (not boots) and ordinary street clothes without overcoat, raincoat, handbags, or packages. The exact reading of the scale should be recorded as the weight and no deductions or adjustments made by the weigher for clothing or other reasons.

5. All original weights should be reported, giving the calculated average weights for each sex and age group and the location where weights were taken and recorded.

6. The report should state the known or estimated population of each community and the number of individuals weighed in each sex and age group.

7. It is desired that the weights be obtained each month at locations approximately identical with those previously chosen.

Yardsticks

Objective measures of nutritional status, such as weight and height for age and sex must be evaluated against some standard, or by serial measurements as discussed previously. Food intake of populations must also be evaluated against some standard. In an effort to provide some orientation as to what can be expected at various levels of calorie intake, table 4 has been prepared. All figures are calories as consumed.

It must be emphasized that there are all gradations between the levels selected. Furthermore, many factors will influence the adequacy of any specific level such as the prior nutritional history of the population, rationing schemes, activity level, environmental temperature, average body size, population composition, and time. The figures presented are for calories only. It is obvious that the calories must come from foods which also will provide sufficient quantities of proteins and other nutrients. The figures presented are derived from practical experience more than from precisely determined values (*7*, *11*, *25*, *34*).

TABLE 4

EXPECTED EFFECTS OF VARIOUS CALORIE LEVELS

Daily calories	Expected results in population
2, 400	Sufficient to maintain health, work output, morale, and body weight over long periods; growth of children normal; normal incidence of signs related to nutritional deficiency.
2, 000	Sufficient to prevent unrest and distress for at least six months; suboptimal work output; some loss of body weight in heavy workers; morale fragile; children and other vulnerable groups can be protected with differential rationing; few signs of nutritional deficiency.
1, 600	Population can plan only for survival unless some groups are deliberately starved to provide for others; morale drops to low levels; sustained work output impossible except with loss of body weight up to 25 percent; growth of adolescent children may be retarded; signs of nutritional deficiency appear.
900	Purely survival activities possible for a few months; no appreciable work output; famine edema appears in as many as 20 percent of older adults; loss of body weight averaging 15 to 20 percent, in severe cases up to 45 percent of body weight; cases of outright starvation are encountered; mortality rates increased; menstrual irregularities in 50 percent of women; diarrhea and nycturia in 25 percent of population; growth of children retarded.

Another yardstick frequently applied is that serious effects from malnutrition will not be found until individuals begin to lose weight to 10 percent or more below their ideal weight. Davidson *et al.* in Vienna in 1945 found 48 percent of the adults to be below U. S. standards by 10 kg. or more. The mean weight loss was 9 kg. per adult (*9*). During the peak of undernutrition in western Holland in 1945 the body weight loss of adults averaged 15 to 20 percent (*7c*).

A yardstick of importance is: How much must a ration be increased to produce a given response in body weight? In other words, what is the calorie cost of producing 1 kg. of body weight gain in adults? Berry has examined the limited amount of data which are available (*4*). Obviously a precise figure cannot be given since activity and energy expenditure increase as caloric intake changes toward normal. Also, it is well known that a given change, for example a 200 calorie daily increase, does not lead to a continuing gain in body weight.

Rather there is a period of weight gain followed by weight stabilization at a new level. The converse of this is also true when calories are reduced. Berry estimates that from 125 to 300 calories daily are required to produce and maintain 1 kg. of weight gain in adults. This figure will vary considerably at different planes of nutrition. These figures are preliminary but do provide a guideline for food planning.

Summary

Certain vital statistics, in particular, crude death, infant mortality, and tuberculosis death rates, can be used to provide presumptive evidence of the nutritional state of a population. National food supply figures are useful if the "normal" food supply and the composition of the population are known. In nearly all instances, reliable assessment of nutritional status requires a direct examination of an adequate cross section of the population. The sample selected can be assessed by combining medical examinations, diet histories, and height-weight determinations. Weight of adults in relation to height, age, and sex is probably the most sensitive, reliable, and objective of the available methods, particularly when periodic observations of the same group are made.

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Clinical Examination

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The clinical examination for the evaluation of the nutritional status of population groups may vary from simplicity itself to one of great complexity depending upon the purpose, and upon the time, personnel, and special equipment available. As a general rule in nutritional status surveys both specialized equipment and detailed observations should be sacrificed to only significant observations in a greater number of subjects. For example, should one wish to know whether or not a particular group is receiving sufficient calories to maintain weight, it is only necessary to do serial weighings on an adequate sample of that population as presented in the previous paper by Sebrell and Hundley. If the population is losing weight, the calories are insufficient. If, for example, over a 10-week period they have lost 2 pounds per caput then by metabolic calculations one can surmise that the diet has been short by about 100 calories per caput per day (5, 7).

On the other hand, should one wish to know if an increased nutrient intake has resulted in clinically detectible changes in the nutritional status, a more extensive examination becomes necessary. Let us presume that flour enrichment with thiamine, niacin, riboflavin, and iron is to be introduced. Then one would have to examine for the significant clinical signs for each of the enrichment nutrients, and if possible, for a control nutrient not supplied by enrichment. After one or more years, the same observations should be repeated in the same season of the year. Significant changes in the frequency of the clinical findings then must be interpreted in the light of changes in nutrient intake attributable to education and economic factors, as well as to enrichment. It is to aid in this evaluation that a control nutrient should be included whenever possible in the assessment of the clinical status when the purpose is to evaluate changes resulting from specific measures such as cereal enrichment.

I have mentioned as a general rule that both specialized equipment and detailed observations should be sacrificed to only significant observations in a greater number of subjects. For example, in a recent survey to determine the effectiveness of flour enrichment the survey group originally included a clinical examination that would have required a minimum of 1 hour, plus an additional hour for a detailed history, plus another additional hour for obtaining various blood and urine specimens and for various special examinations such as slit lamp, dark adaptation, electrocardiograms, and chest X-ray. With experience they might have examined 8 persons per day, and with 1 clinical medical examiner after a month in the field have observations on a maximum of 200 persons. The drudgery of tabulating and statistically analyzing this mass of data is depressing for the value that could possibly be obtained from all various groups in a general population from a sample of only 200 persons. By persuasion the time was halved by deleting certain minutiae, and 400 persons were examined but the survey would have benefited still more by further drastic cutting of additional minutiae so that 800 or more persons could have been studied.

How may one assess nutritional status of population groups when it is well recognized (*6*) that each of the clinical signs is nonspecific? How can such clinical signs be regarded with confidence when it is warned that each clinical sign must in the individual be interpreted in the light of the history, of other clinical signs, of laboratory data, and sometimes only after a therapeutic test (*6*)? For example, angular stomatitis is a sign of riboflavin deficiency but it will also occur as a result of ill-fitting dentures, antibiotic administration, and iron-deficiency anemia. Red swollen bleeding gums is a sign of scurvy but it may occur from mechanical factors, local and systemic infections, leukemia, as well as from deficiencies in niacin and vitamin K. Tongue lesions characterized by redness and by papillary hypertrophy followed by atrophy occur in niacin deficiency but they also occur in vitamin B_{12} and folic acid deficiencies, protein deficiencies, and sometimes as a result of dentures. Follicular keratosis occurs as a result of vitamin A deficiency but follicular involvement occurs as a result of acne vulgaris, dirt and some chemical agents used in industry. In addition, the apparent folliculosis of severe calorie deficiency or of adolescence must not be overlooked. Dependent edema is a sign of protein deficiency when severe enough to cause hypoproteinemia, but it is also a sign of congestive heart failure, sodium retention from any cause, starvation, and beriberi. It is common during pregnancy and in extremities following frostbite. Absence of the Achilles tendon reflex bilaterally is a sign of beriberi, but this absence also occurs in a variety of other neuropathies and lower motor neuron lesions.

Overweight for height as judged by tables of desirable weights is a sign of obesity but it may also occur as a result of fluid retention or by greater muscular development than the average. Skin thickness as measured by the Minnesota calipers[1] or by a simple pinch test (*5*) is probably the most reliable single sign of obesity, other than underwater weighings which are hardly practical for survey purposes. Yet, in spite of this apparent simplicity, the calipers must be correctly used and skin edema and several more or less rare skin diseases will cause either the skin or its attached subcutaneous tissues to hypertrophy or to atrophy and cause deviations important in the individual.

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[1] Skin calipers designed by Ancel Keys.

These facts of nonspecificity in any individual subject and the lack of individual interpretation in surveys does not make it impossible to use certain of these signs as indices of malnutrition in population groups. Even so, quantitation may be attempted by deducting a figure that includes an estimate of the frequency of the signs due to non-nutritional factors. As a matter of record, the clinical examiners of the Newfoundland survey group in 1944 and 1948 (*1*, *2*) were able to predict from the clinical findings the results of the chemical examinations many weeks before these were available. In 1944 it was with considerable hesitation, but with that experience behind us, the prediction on the basis of the clinical signs in 1948 was made with some confidence.

Let us examine the evidence that certain clinical signs of malnutrition may be used as an index of nutritional status in surveys. In Newfoundland in 1944, as shown in table 1, the intake of riboflavin was approximately 1 mg. per caput per day; 30 percent of the subjects excreted less than 200 μg. of riboflavin per gm. of creatinine; 21.7 percent had angular stomatitis; 26.2 percent had significant cheilosis; 46.4 percent had hyperemia of the conjunctivae; and 10.7 percent had a magenta tongue. In 1948 the intake of riboflavin was about 2 mg.; 1 percent of the subjects excreted less than 200 μg. of riboflavin per gm. of creatinine; angular stomatitis was now 9.7 percent; cheilosis 6.2 percent; 23.2 percent had conjunctival hyperemia; and 0.3 percent had a magenta tongue.

TABLE 1

RELATION OF CERTAIN CLINICAL SIGNS TO RIBOFLAVIN DEFICIENCY IN NEWFOUNDLAND

	1944	1948
Intake of riboflavin per caput per day	1.0 mg	2.0 mg.
Excretion of riboflavin in urine per gm. of creatinine. Less than 200 μg.	30.0 percent	1.0 percent.
Angular Stomatitis	21.7 percent	9.7 percent.
Significant cheilosis	26.2 percent	6.2 percent.
Conjunct. hyperemia	46.4 percent	23.2 percent.
Magenta tongue	10.7 percent	0.3 percent.

On the basis of these findings, it is suggested that angular stomatitis be considered the key sign of riboflavin deficiency in clinical surveys on the basis that its presence or absence is subject to less personal interpretation than the other mentioned signs. These other signs should be looked for and recorded, however, for when they also occur in a high frequency in a population group one can feel more certain that the key sign is a fair index of the prevalence of riboflavin deficiency. If angular stomatitis should occur in a high percentage of a population group with an insignificant incidence of these other signs, it is probable that the angular stomatitis is on a basis other than that of riboflavin deficiency.

Table 2 shows that in Newfoundland in 1944 the intake of vitamin A was about 1,500 units; 48 percent of the group surveyed had less than 20 µg. percent of serum vitamin A; follicular changes on the arm or legs was about 42.3 percent and on the face about 7.5 percent. In 1948 after fortification of margarine with vitamin A sufficient to double the per caput intake of vitamin A to about 3,000 I. U. only 2 percent of the subjects now had serum vitamin A below 20 µg. percent and follicular changes had decreased to 27.1 and 2.3 percent, respectively, on the arms or legs and on the face. It is thus suggested that follicular keratosis over the back of the arms can be used as the key clinical sign. Other localities can be used for supporting evidence, the best one being over the buttocks, but that is not practical in mixed population surveys. The face is probably the most accessible, but here one must not confuse the folliculosis of a vitamin A deficiency with acne. As a matter of fact, folliculosis is so high during adolescence that extreme caution must be observed particularly in the temperate zones during winter. Spot lesions in the conjunctivae might also be used as a supporting sign, but in Newfoundland all grades of thickening were tabulated together making our findings there not particularly useful for this purpose.

TABLE 2

RELATION OF CERTAIN CLINICAL SIGNS TO VITAMIN A DEFICIENCY IN NEWFOUNDLAND

	1944	1948
Intake of vitamin A per caput per day	1,500 I. U.	3,000 I. U.
Serum vitamin A less than 20 µg. percent	48 percent	2 percent.
Follicular changes arms or legs	42.3 percent	27.1 percent.
Follicular changes face	7.5 percent	2.3 percent.

Unfortunately, we have no chemical data for niacin in the Newfoundland surveys. We do know, however, that in 1944 the per caput intake of niacin was borderline, and the enrichment of flour would increase this figure by about 8 mg. per day. There was in 1948 a statistically significant decrease in the following tongue lesions: hypertrophic papillae at the tip and elsewhere, atrophic papillae at the tip, and atrophic papillae at the tip and elsewhere (see table 3). Hypertrophic papillae at the tip only were unchanged and a reddened tongue dropped in frequency but not sufficient to be statistically significant. Furrowing or fissuring was unchanged. These figures combined with our information concerning tongue changes in pellagra, sprue, and pernicious anemia would suggest that papillary change more advanced than papillary hypertrophy at the tip of the tongue is the key clinical sign in this instance.

TABLE 3

RELATION OF CERTAIN CLINICAL SIGNS TO NIACIN DEFICIENCY IN NEWFOUNDLAND

	1944	1948
Approximate niacin intake per caput per day	10 mg	18 mg.
Hypertrophic papillae at tip and elsewhere	21.1 percent	7.7 percent.
Atrophic papillae at tip	13.9 percent	3.8 percent.
Atrophic papillae at tip and elsewhere	18.4 percent	3.9 percent.
Reddened tongue	5.8 percent	2.4 percent.[1]

[1] Not statistically significant (p> 0.01).

It is indeed unfortunate that the results of the Newfoundland (*1*, *2*) surveys and the Bataan (*3*) surveys were not reported so that the results on thiamine could be better compared. We do have, however, one point of comparability (table 4), namely, the mean excretion of thiamine in the urine per gm. of creatinine. Before enrichment the mean excretion of thiamine in the urine in Newfoundland was 117 µg. as compared with 65 µg. in Bataan. This is compatible with the findings of about 10 percent frank beriberi in Bataan with less than a possible 2 percent maximum in Newfoundland.

TABLE 4

RELATION OF CERTAIN CLINICAL SIGNS TO THIAMINE DEFICIENCY IN NEWFOUNDLAND AND BATAAN

	Newfoundland		Bataan	
	1944	1948	1944	1948
Approximate thiamine intake per caput per day.	0.96 mg	1.8 mg	0.7 mg.	1.9 mg.
Excretion of thiamine in urine per gm. creatinine. Less than 50 µg.	44 percent	1 percent	----------	----------
Mean excretion	117 µg	297 µg	65 µg.	253 µg.
Absence of knee and ankle jerks.	1.8	1.3 [1]	----------	----------
Calf muscle tenderness	2.0	1.0 [1]	----------	----------
Frank beriberi [2]	----------	----------	9. 9	0
Suspected beriberi [3]	----------	----------	25. 7	0

[1] Not statistically significant (p>0.05).

[2] Three signs or 2 signs plus 1 symptom.

[3] Two signs and no symptoms or 1 sign and 2 symptoms.

It is suggested as a screening test for beriberi in clinical nutrition surveys that only one key test be done, namely, the Achilles tendon reflex. The diagnosis of peripheral neuropathy of the type occurring in beriberi could not be made in its presence (*4*). Then, if absent, that person should be pulled out of line and further examination made to classify the patient as "frank beriberi," "suspected beriberi," and "no beriberi." Those whose ankle jerks are present are routinely considered as belonging to the "no beriberi" group.

Table 5 showing the relation of various clinical signs of ascorbic acid deficiencies with blood levels in itself does not support any of these signs as an index of vitamin C deficiency. However with similar findings in New York State of a 1.3 percent incidence in school children of red hyperemic gums when the blood levels are approximately double that in Newfoundland, and an incidence of about 5 percent in a school in Havana where the intake of ascorbic acid is relatively high, being about the same as in New York State, it is felt that this sign should be used as the key sign of ascorbic acid deficiency. Personally, I feel that any degree of marginal redness should be recorded and any figure greater than 5 or 10 percent prevalence as indicating some degree of ascorbic acid deficiency in the population surveys. Perifolliculosis can be used in an edentulous population, but here its presence in any amount would be significant as this lesion is seldom observed until after the blood ascorbic acid has fallen to zero.

TABLE 5

RELATION OF CERTAIN CLINICAL SIGNS TO ASCORBIC ACID DEFICIENCY IN NEWFOUNDLAND

	1944	1948
Approximate ascorbic acid intake per caput per day.	20 mg	27 mg.
Serum ascorbic acid, mean	0. 49	0. 41.
Less than 0.2 mg	28 percent	28 percent.
Gums, red hyperaemia, grade 1	25. 8 percent	35. 5 percent.
Grade 2 and greater	14.8 percent	14.1 percent.
All grades	40.6 percent	49.6 percent.
Swollen gums	45.2 percent	53.1 percent.
Perifolliculosis	2.0 percent	6.6 percent.

In table 6 are tabulated the various signs that can be recorded in a clinical nutritional survey. The underscored signs are the key signs. The others are supplementary or confirmatory signs. These signs are of course the minimum necessary for a general clinical nutrition survey, but additional ones should be included only when the necessity for additional clinical data is clearly evident before routinely observing them in a general nutrition status survey.

There are several nutrients, including iron, calcium, pyridoxine, B_{12}, and folic acid that are not mentioned. Iron and calcium are omitted because there are no good early clinical signs which can be made to correlate with the facts; for iron deficiency we must depend upon the laboratory and for calcium deficiency upon the X-ray. I know of no good dependable key clinical sign for use in surveys for pyridoxine, folic acid, and B_{12} deficiencies outside their classical clinical syndromes, although it might be possible to use a reddened tongue and diarrhea in an area where sprue is frequent as an index of folic acid deficiency in a clinical survey.

TABLE 6

Nutrient	Clinical sign	Abnormal
Calories:		
Obesity	*Skin thickness:*	
	Scapulae	Over 30 mm.
	Lower Axillae	Over 25 mm.
	Height-weight tables	Over 10 percent.
Leanness	*Skin thickness:*	
	Scapulae	Under 8 mm.
	Lower Axillae	Under 8 mm.
	Height-weight tables	
Protein	*Dependent edema*	Over 0 percent[1] under age 50. In absence of beriberi starvation, and pregnancy.
Vitamin A	*Follicular Keratosis* of arms	Over 5 percent[1] in adults and pre-adolescents. Not reliable in starvation and in adolescents.
Vitamin D	Under six months *Craniotabes*	Over 0 percent.[1]
	Six mo. to two years *Beading of the ribs.*	Over 0 percent.[1]
	Over two years ( ) *and X deformities of legs.*	Over 0 percent.[1]
Thiamine	Absent Achilles tendon reflexes.	Over 1 or 2 percent.[1]
Niacin	*Tongue lesion more advanced than hypertrophy of papillae at tip.*	Over 5 percent.[1]
	Reddened tongue	Over 1 or 2 percent.[1]
	Pellagrous dermatitis	Over 0 percent.[1]
Riboflavin	*Angular stomatitis*	Over 5 percent[1] in non-denture wearing population.
	Conjunctival hyperemia (circum corneal infection).	Over 5 percent.[1]
	Magenta tongue	Over 0 percent.[1]
Ascorbic acid	Red hyperemic gums	Over 1 or 2 percent[1] in children. Over 5 to 10 percent[1] in adults.
	Perifolliculosis	Over 0 percent.[1]

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[1] An educated guess of frequency that these signs might occur in a well-nourished population group.

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FRIDAY AFTERNOON SESSION

26 February 1954

DR. HERBERT POLLACK, *Presiding*

Physical Performance[1]

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Introduction

There would be little disagreement, I believe, about the proposition that the final criterion of a football player's "fitness" is his performance in a football game or, to reduce the effect of "chance," in a series of football games (*55*). Similarly, in the industrial situation the level of output is a pragmatic measure of performance capacity. In the field of military operations the skill with which the pilot handles his plane during combat, the endurance of the infantrymen during a long march, the speed of movements and strength in a hand-to-hand clash, the sustained alertness of a man on a lonely watch post or the rapidity and accuracy with which the movements of an enemy submarine are charted on the basis of data taken down from the radar scope—these and thousands of other operations are the final and irrevocable criteria of fitness.

The very multiplicity of the operations involved in modern industry and warfare is the first stumbling block in any attempt to examine the relationship between performance and nutrition. Experimentation in which the actual performance is used is, as a rule, not feasible. Furthermore, in most operations the information on the quantifiable aspects of performance is lacking and is not readily obtainable. Consequently, the reduction of the variety of actual tasks to a small number of prototypes and basic functions, which constitute the limits of performance, is imperative. Thus, theoretically at least, in studying performance in the laboratory the experimenter has the choice of using either simplified performance tests (miniature work situations) or the more abstract tests of the biological components of performance capacity.

In military ration tests attention has been paid principally to the biochemical and physiological (cardiovascular) characteristics. "Morale" and physical well being have also been considered, mostly without the use of systematic and standardized questionnaires. Per-

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[1] Supported, in part, by Quartermaster Contract No. DA44-109-qm-1526.

formance in long marches would appear as an excellent test of endurance but it has been found repeatedly that the condition of the feet, having little to do with nutrition, proved to be the major factor in capacitating the men. While the problem of quantitative characterization of the on-the-job performance is frequently undeniably difficult, in many operations it is not insuperable. A good deal of attention has been paid this question in the framework of Army personnel research in reference to selection and training (*32*). For some types of military performance it is possible to use scores characterizing quantitatively achievement in a standardized field situation. Thus Tyler (*67*) utilized data obtained on the rifle firing range in his study of the potential impairment of skilled performance resulting from the administration of drugs designed to prevent motion sickness. We are not aware of a systematic use of such criteria of performance in nutritional laboratory or field studies.

The two types of tests of physical performance which had been applied intensively in laboratory research—riding on a bicycle ergometer, and running or walking on the treadmill—cover only a small sector of operations found in modern industry and the Armed Forces. In addition to locomotion, which represents gross physical performance, a test battery designed to evaluate comprehensively the various components of performance capacity should cover also manipulation (psychomotor tests), perception, and intellective functions. The concern with personality in the framework of performance studies is justified directly by the crucial role of motivation in performance, the relationship between personality structure and stress tolerance and, indirectly, by the fact that some types of performance can be used as a basis for personality assessment.

Two facts must be taken into account if performance tests are to yield valid information: (*a*) Performance (P) represents a product of capacity (C) and motivation (M)—$P=C\times M$, and (*b*) scores in many of the performance tests are susceptible to improvement with practice. These facts place rather unique and heavy demands on the experimenter who must carefully define his control measurements. Such factors as presence of other subjects in the room or the subject's knowledge of the scores affect the level of effort, and "random" variations in the human environment should not be allowed to complicate the motivational structure of the experiment. Systematic studies on motivation of laboratory subjects are nonexistent. Fraser's study (*19*) has indicated that presence of the experimenter served as an additional stimulus, decreasing the number of errors in a prolonged visual task.

In fundamental investigations on nutrition and fitness, where we are concerned with the amount of potential deterioration of the functional efficiency of the human organism under the most severe form of the given nutritional stress, the technique of multiple tests covering the whole spectrum of the components of performance capacity represents the desirable approach. In studies in which we deal with the differences between the effect of different levels of nutritional deficit, only those criteria of fitness which were proved to be sensitive to the particular type of nutritional stress should be used.

Actual Performance

In general, quantitative records of actual performance can be more readily obtained in the industrial than in the military setting and published studies on nutrition and work performance are limited to industrial output. Work output is a very complex datum and uncontrolled factors readily mask or simulate deterioration or improvement in performance capacity which is then erroneously ascribed to the diet. While from the point of view of a "realistic" appraisal, the output figures have an undeniably strong appeal, the ready distortion by extraneous factors should be kept in mind. This is true even when we are concerned with the total impact of nutrition, not only with "performance capacity." It has been noted that the work output can be interpreted as a decrement in capacity only when effort is kept "constant." While the scores in performance tests carried out in the laboratory with highly motivated subjects approach the limit of their capacity, the intensity of industrial work is always submaximal. The wider the gap between capacity (the physiological limits of performance) and the actual output, the more important is the effect of the uncontrolled physiological and the variable psychological and socioeconomic factors (*5*).

The danger of neglecting the uncontrolled, non-nutritional factors is well illustrated in the pioneering study of Haggard and Greenberg (*22*) on the relationship of the frequency of meals and output in a plant manufacturing rubber footwear. The operation selected for study involved sewing the canvas parts of tennis shoes. For this operation it was easy to keep individual hourly production records and there was no interference with production due to supply of material. The workers operated power-driven sewing machines and in terms of energy expenditure the job corresponded to light manual work. The design of the experiment appears to have been carefully planned, with the 40 operators divided into an experimental and a control group. The latter group ate 3 meals throughout, the experimental group alternated every 2 weeks between 3 and 5 meals. The two additional meals were supplied without cost and consisted of a glass of milk and a 6-ounce piece of angelfood cake. The extra meals were served at the beginning of the third hour of the morning and afternoon work period; apparently there was no similar "break" on the days when only three meals were eaten. The mean hourly production of the control group varied minimally (between 183 to 194 units) during the five 2-week periods. The output of the experimental group was consistently higher during the second and third period when the workers were given the additional meals, with mean scores of 175, 192, 176, 194, and 176 units during the five periods.

The fact that the production was raised on the between-meal-feeding schedules appears to have been clearly established. Unfortunately, the experimental conditions do not allow a clear-cut identification of the factors responsible for the increased output. Specifically, the rest pauses alone, provided by the additional meals, could account for all or part of the effect. Also, the psychological impact of the additional meals on the workers' morale (motivation), which may be independent of the nutritional value of the extra meals, could not be separated from the direct, physiological improvement—if any—in the work capacity. The physiological interpretation of increased production through high muscular efficiency maintained by more frequent meals was open to question (*5*), but the principal need was for additional data on the effect of added meals which varied in the nutritional content.

Such data were supplied by Haldi and Wynn (*23*). The operation resembled, in principle, the type of industrial work studied by Haggard and Greenberg, and involved sewing cotton bags. The 4 types of meals, served for 3 consecutive weeks during a 15-minute period in the mornings and in the afternoons, provided about 650, 300, 150, and 80 calories, respectively. The order of serving these four types of refreshments was varied each week. Once a day during 2 successive weeks no food was given during the rest periods.

Thirty-two seamstresses served as volunteer subjects and their hourly outputs were determined. Combining the morning and afternoon values, the pre-refreshment outputs on days at which the 4 types of refreshments were given were 124.0, 122.5, 123.5, and 122.5. The corresponding post-refreshment averages were consistently higher (136.5, 133.5, 134.0, and 133.5 percent of the standard hourly production) but there were neither statistically significant nor practically important differences between the average values obtained for the 4 dietary regimens. It was concluded that in this type of industrial work the output is not affected by the kind and amount of supplementary food eaten during the midmorning and afternoon rest periods. While the additional meals had a definitely positive influence on the rate of production during the period under observation, no definite conclusions on the long-term effects and the maintenance of such a differential in output before and after the food refreshments could be drawn on the basis of available data.

Kraut and Müller (*38*) reported investigations on industrial output as a function of calorie intake. Data on food consumption, work performance and body weight of workmen who were removing debris out of railroad cars by hand, of steelworkers, and of several groups of miners were interpreted as indicating that the workers tended to adapt, perhaps unconsciously, the energy expenditure to the available food supply, keeping the body weight relatively constant. The parallelism between calorie consumption and work output was by no means perfect and was distorted, at least for a time, by variations in motivation, non-nutritional in origin. Thus the institution of an incentive system, with cigarettes as the bait, substantially increased the output over the previous level while at the same time the workers lost, on the average, 3.5 kg. of body weight in half a year.

Miniature Work Situations

In a variety of sectors of the study of human work it has been found useful to employ standardized, simplified replicas of an actual industrial or military performance. Their uses in studies on general and on occupational fitness, the application of synthetic training devices, and use of miniature work situations in the study of fatigue (*14*) have been reviewed (*11*). The review was prepared as a report to the Subcommittee on Physiological Stress in Industry (Committee on the Nutrition of Industrial Workers, National Research Council) in the hope of drawing attention to the usefulness of this technique in fundamental and applied studies on performance and work capacity and their deterioration under a variety of physiological stresses. Mackworth (*40*), with encouraging results, used a number of synthetic work tests in his studies on detection of faint visual and auditory signals indicating the presence of the enemy and in his researches on human performance in abnormal atmospheric environments (*42*).

The miniature work situations represent a potentially useful tool also for nutritional research. They are a compromise between the realism of the complex, not rigorously controllable operations in an industrial plant or a military theater, and the schematic performance tests applied in the laboratory. As far as we are aware, no systematic use has been made of this techinque in the evaluation of the effect of nutritional factors on performance.

In a study incidental to the examination of the effects of the duration of visual work and the illumination intensity (*12*), visual performance and a number of visual functions were considered in reference to the composition of a noon meal (*61*). The miniature work test reproduced the operation of inspecting objects, transported on a conveyor belt, for small details.

While the synthetic work situations have undeniable advantages in research on sensorimotor functions, the disadvantages and limitations should not be minimized. Their construction and application are usually costly and the tests are likely to occupy a sizable amount of the laboratory space. In longitudinal experiments, typical of nutritional studies, the improvement with practice may present a difficult problem. Most importantly, perhaps, the very specificity of the tasks, which makes the results applicable to the concrete situation of which the test is a replica, limits the possibilities of generalization. These considerations place the componental study of performance capacity in a better light than it would appear to merit in the light of theoretical analysis of manipulative and perceptual skills (*6*).

Laboratory Tests

Excluding the miniature work situations, which usually involve more complex types of performance and in which the score represents an integral of several functions, the more classical, analytical tests may be grouped under the headings of locomotion, manipulation, perception, and intellective functions. Manipulative tests, with strength, speed, and coordination of movements as different aspects of manipulation, are frequently referred to as psychomotor tests. At times, only speed and coordination have been included in this category, and strength set apart. While the factors limiting performance are certainly different, running on the treadmill to exhaustion is no less "psychomotor" than tapping at a maximal speed. Endurance is a separate aspect of performance rather than a separate function. It is very difficult or impossible to devise satisfactory laboratory tests of endurance, unless one is using tasks in which exhaustion is reached in a matter of minutes. Yet it is the prolonged work of low to moderate intensity of energy expenditure which is characteristic of the overwhelming majority of industrial jobs. Even in the military operations the capacity for strenuous physical work is critical in a surprisingly small number of operations.

A brief but fairly comprehensive description of performance tests used at the Laboratory of Physiological Hygiene has been presented (*35*). Other types of muscular performance used in nutritional researches include such tasks as swimming, heavy weight lifting, and pulling wall weights (*30*), endurance in horizontal pulling of submaximal pulley weights, endurance in holding a weight with an outstretched arm, and decrement in force on repeated exertion of maximal muscular force (*62*).

Where we deal with larger groups, with good opportunity for provision of rigorous control observations, and are concerned with rather gross changes in performance capacity, the use of standardized athletic tests, such as push-ups and sit-ups, might be useful. The use of the length of time for which a child can hang from a bar should be mentioned for the purpose of completeness rather than because it is considered a valuable measure of performance capacity. Jokl (*41*) measured physical efficiency as the time of running 100 yards (speed) and 600 yards (endurance), and the distance in shotput (strength). On comparing the performance of children on a normal dietary regimen and during the Ramadan fast, the most consistent decrements were noted in the first and third tests. On the other hand, in the presence of vitamin C deficiency no impairment of muscular performance was observed.

Motor Performance

Locomotion.—The principal type of motor performance which has been studied by the physiologist is locomotion, either in the form of riding a bicycle ergometer or walking and running on a motor-driven treadmill. Dealing with performance aspects of physical exercise, one may be concerned with the speed, endurance, or infrequently, with coordination evaluated on the basis of oxygen consumption in a standard task. In the endurance tests the speed (and other conditions constituting the work load, such as the resistance against which the subject is pedalling or the incline of the treadmill) is fixed and the score is measured in terms of time of work to the point of cessation of the activity due to exhaustion. In bicycle riding the exhaustion point is frequently determined as the moment at which the subject is no longer able to maintain the predetermined rate of pedalling (*30*). Or one may keep the time constant and determine the speed at which the subject is capable of carrying on the task for a specified time. The latter form is of greater interest for exercise physiology (*2*) than for the study of nutrition.

Physiologically, physical work has been classified into two types: (*a*) anaerobic (severe exertion of short duration, inadequate oxygen supply to the muscles, and increasing level of lactic acid in the blood), and (*b*) aerobic (can be carried on for longer periods, adequate oxygen supply, a steady state of respiratory and circulatory functions, no increase in the level of the lactic acid in the blood beyond the initial period of adaptation to work). These two types of work involve different mechanisms by which the energy needed for muscular contraction is released. They produce different physiological responses, and exhaustion (fatigue) depends on different factors. Consequently, both types of work should be included in a comprehensive battery of performance tests.

As measures of endurance, anaerobic tests are easy to administer in the laboratory as the times involved are measured in seconds and minutes. Introduction of really exhausting aerobic work for this purpose is difficult, both on account of the long period of time and the problem of morale. Treading on a moving rubber belt for long hours is not a very inspiring job, and the work in the field (marching) provides much more realistic conditions of scenery and motivation.

In the nutritional researches physical work has, as a rule, been studied as an imposed stress, not as a direct criterion of performance. The use of the time of the run in a standard exhausting exercise (*29*) represents inroads of motivated behavior (effort) into the physiologist's armory of objective tests. The overall test score relates the time of run on the treadmill to recovery pulse rate. The duration of effort before exhaustion was given prominent place in the computation of the index of fitness for hard work because "endurance or 'staying power' in man has always been highly esteemed and is an indication not only of stamina, but also of strength, skill, and determination" (*29*). From the point of view of performance studies it is unfortunate that the upper limit of the time of the exercise has been set at 5 minutes. It means that the time scores in a group of men who are in fairly good physical shape have a nonnormal distribution which is difficult to handle statistically. Also, the amount of deterioration of performance under stress and the degree of recovery on nutritional rehabilitation cannot be satisfactorily assessed.

In this context we shall not be concerned with physical work and exercise as an experimentally imposed stress allowing the evaluation of the metabolic, circulatory, and respiratory functions as a criterion of the organism's fitness. This has been one of the physiologist's principal methodological contributions to nutritional research. The methods are well established (*26*) and a detailed discussion of the topic would exceed the framework of this presentation.

Manipulation.—While the biological components or aspects of manipulative performance are few in number—strength, speed, and coordination—the ways in which they may be measured by psychomotor tests are many. Selected methods useful in medical research have been brought together under Miles' editorship (*44*). The voluminous report on the use of apparatus tests in the Aviation Psychology Program of the Army Air Forces During the Second World War (*43*) is another useful source book of psychomotor tests.

The battery of psychomotor tests, developed at the Laboratory of Physiological Hygiene and applied in the study of nutritional and other stresses, has been described (*9*, *15*). It included 2 tests of strength, 3 tests of speed, and a test of eye-hand coordination. Four of these tests are in figures 1 to 4.

Strength was measured by standard hand-grip and back-lift dynamometers. The speed of small repetitive movements was determined by a two-plate tapping test. There was a test of the speed of coordinated hand and arm movements. Reaction time, associated with a selective response to visual stimuli and involving several muscle groups, was determined. Tracing a narrow, grooved pattern with a stylus was used to measure eye-hand coordination. Both the number of the error contacts between the stylus and the sides of the groove, and the total duration of the contacts were used as a criterion of performance.

In addition to the tests included in the standard battery, a variety of other psychomotor measurements has been applied in the Labora-

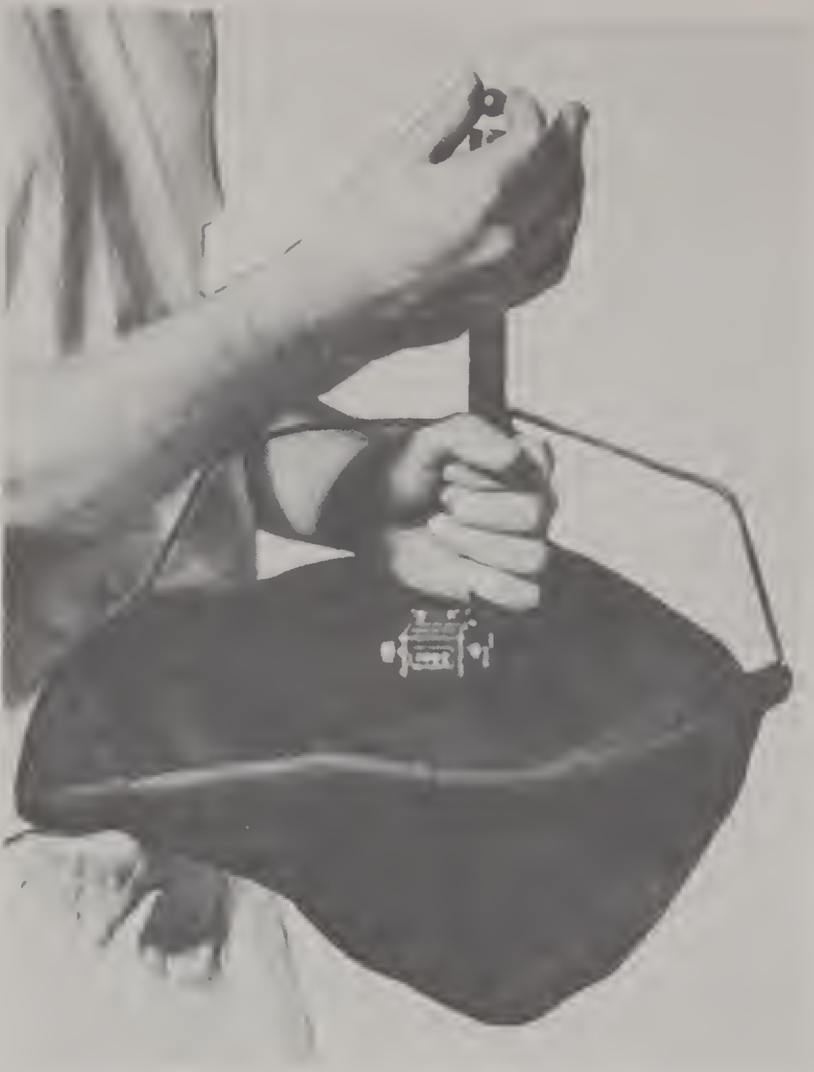

FIGURE 1.—Test of the speed of complex movements involving the pronation and supination of the forearm combined with a vertical displacement of the hand. Score: number of times the ball bearing was passed through the pipe in 60 seconds.

tory (toe reaction time; speed of nonrepetitive leg and arm movements (*13*); and a measure of body sway).

The separation of psychomotor tests into the three categories is useful from the point of view of the presentation of the results, but strength, speed, and coordination are abstract categories. Statistically, the low correlations between tests which belong logically in the same category indicate the high specificity of the motor functions. Physiologically, the factors limiting the speed of different types of movements may have little or nothing in common. Thus in small repetitive movements, e. g., in tapping, the limiting factor appears to be the refractory period of the motor centers; the speed of single ballistic movements depends on the force developed during isotonic contraction; and the reaction times, also a measure of speed, reflect the delay at the central sensorimotor synapses.

It is recognized that the tests in the standard battery used at the Laboratory are not all factorially pure, as some involve more than one abstractly defined function, e. g., speed *and* coordination, and that

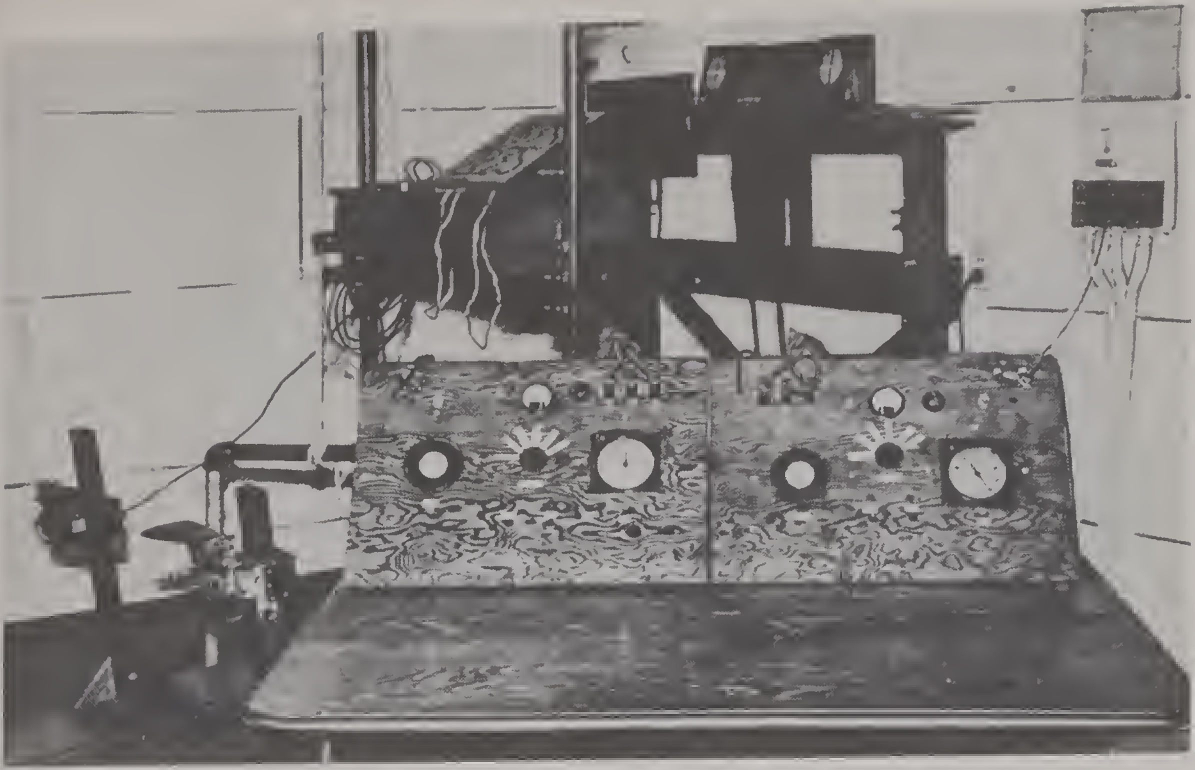

FIGURE 2.—Overall view of the tests of complex reaction time (light signals in the upper middle part—see also figure 3—and large paddles at the lower left corner, to be depressed by the subject walking on the treadmill) and pattern tracing.

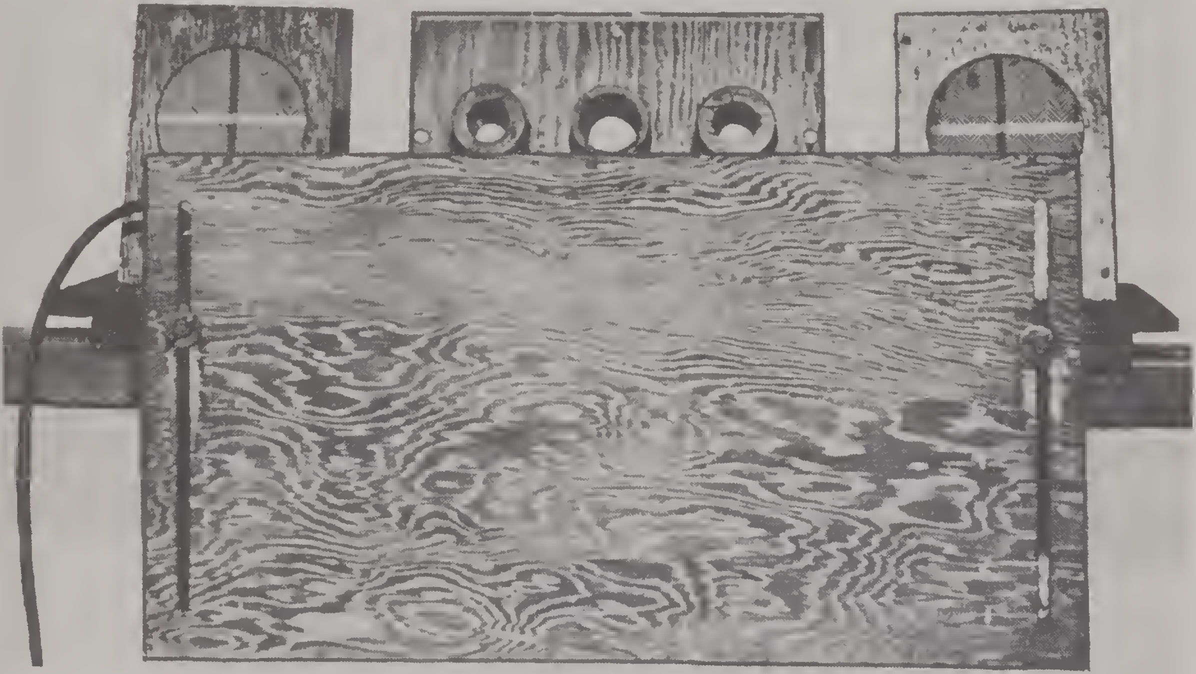

FIGURE 3.—Boxes to the left and right contain a buzzer indicating the subject's errors (contacts) in pattern tracing. Light signals for the reaction-time test are in the center. In the front is an adjustable panel ("blind") the level of which can be adapted to the height of the subject.

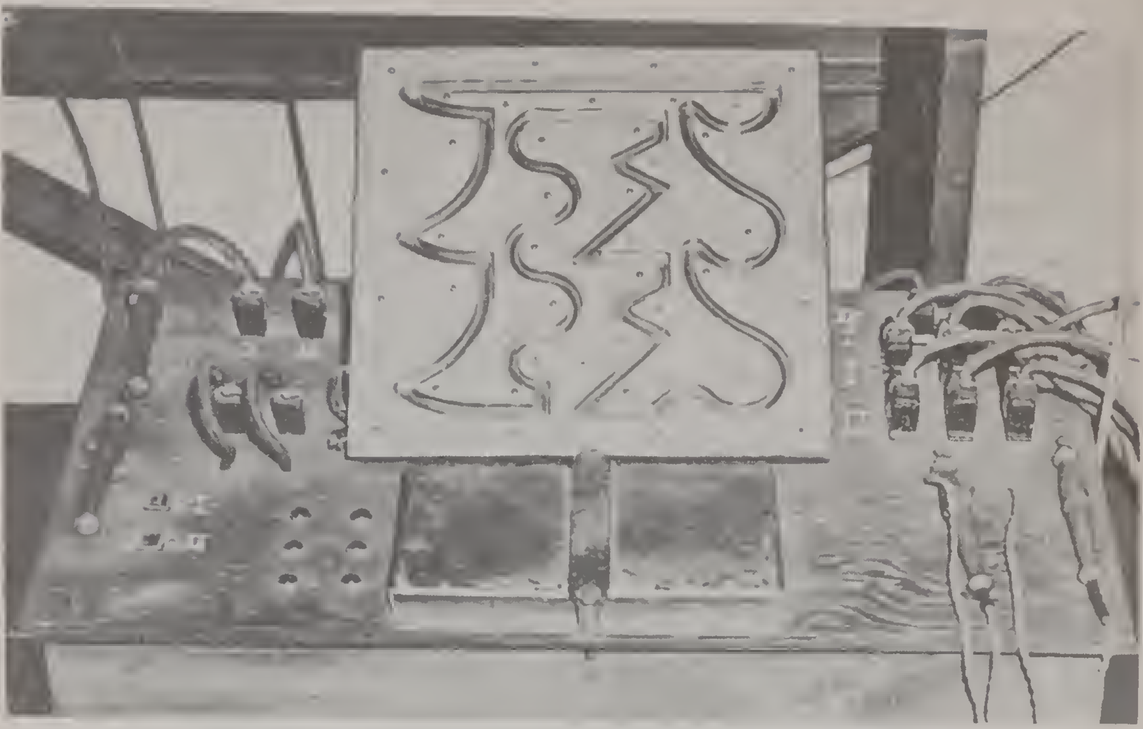

FIGURE 4.—Board with two tapping plates and the tracing pattern. The number of taps in 10 seconds and the number of errors (contacts) made in tracing the pattern with a stylus were used as the performance scores.

the deterioration under stress cannot be safely interpreted in terms of occupational activities. It is through biological validation, through the comparison of changes under a variety of nutritional and nonnutritional stresses (semistarvation, acute starvation, hard work, heat, lack of sleep (*15*)), that the relative decrement in performance can be interpreted in meaningful biological terms.

Sensory Functions

In studying sensory functions as criteria of 1 aspect of performance capacity we are interested in 2 types of functions: (*a*) functions which are known to be affected in a specific way by a nutritional deficiency (deterioration of dark adaptation as a criterion of vitamin A depletion, with a prolonged cone-rod transition time and raised rod threshold (*27*)); and (*b*) general functions which are of importance in the individual's interaction with his environment and which may determine or at least affect his occupational efficiency.

This means that we are concerned with vision and hearing rather than with proprioception, skin senses, and taste and smell, although in particular circumstances almost any mode of sensory perception may acquire occupational importance. In the Laboratory, the study of hearing in reference to nutritional factors has been limited to audiometric determination of the threshold intensity at selected frequencies. From the point of view of military performance the localization of sound in space may be just as important as auditory acuity (for methods see (*69*)).

Vision is a considerably more complex function. In addition to visual acuity in far and near vision, visual fitness involves such functions as the rate of visual perception, color discrimination, and depth perception (Weston (*68*), who described occupational demands upon sight and testing sight for work). Some aspects of visual motor behavior can be used as tests of psychomotor performance (*7*, *8*). While the quantitative analysis of eye movements had not been actually applied in nutritional research, the development of the procedure was stimulated by its potential use in experimentally induced deficiencies of some of the vitamins of the B complex, principally thiamine, in which a marked deterioration of motor performance had been observed (*9*).

Sensory, especially visual perception, plays a crucial role in an ever increasing number of industrial and military operations. Where the potential effect of diet is to be investigated, synthetic tests, such as the auditory and visual vigilance tests used by Mackworth (*40*), may yield more useful information than the abstract tests of auditory and visual acuity which have been used in nutritional studies in the past.

Intellective Performance

Intelligence may be described in terms of: (*a*) "mental level" determined by power tests, administered without time limits, (*b*) performance in speed tests, with strict time limits, and (*c*) learning ability, measured in reference to the amount of improvement in a mental task.

The number of available standardized tests of intelligence and of specific (mechanical, clerical) abilities is large (*16*, *17*, *18*). However, several requirements peculiar to nutritional research (and to the study of other biological stresses, such as heat, which may be superimposed on dietary restrictions) markedly reduce the potential supply of useful tests. In most of the situations in the framework of which the psychometric technique were originally developed—educational counseling, industrial and military selection, psychiatric diagnosis—the testing was aimed at the determination of intellective capacity at the given time. The majority of the intelligence tests have only 1 or at the most a few alternative forms and the testing is done on 1 or a limited number of occasions. In nutritional research it is necessary to test intellective functions at intervals in order to determine the progressive effects, if any, of the particular dietary regimen. In some types of dietary restriction or supplementation the intervals are measured in terms of months. In acute deficiencies and total starvation, and in the study of combined (multiple) stresses the interval between repeated testing sessions is reduced to days and this eliminates just about all the tests of intelligence available on the market.

Because of the time involved in individually administered tests, as a rule, only group tests of intelligence can be considered for the study of intellective functions in relation to nutrition. Most of the intelligence tests involve verbal material. The Porteus' Maze Test was used in nutritional studies as manipulative, nonverbal test of intelligence (*56*, *57*). The applicability of this and other nonverbal tests to non-English-speaking subjects is of importance for field research on diet, behavior and performance capacity in native populations suffering from severe nutritional deficiencies—a vast, important but barely touched segment of research.

The CAVD scale, consisting of four subtests—Completions, Arithmetics, Vocabulary, and Directions—is a good example of the power tests of intelligence. The items are grouped into 17 levels increasing progressively in difficulty, up to the level of very high abstract intelligence. With relatively homogeneous groups only a small number of such levels has to be supplied. In the starvation study (*35*) we used the 5 highest levels, with a maximal score of 40 points for each level. Most of the subjects took 8 to 10 hours for completing the test, working for about 2 hours at a time. The time-consuming character of the test clearly eliminates its use for nutritional studies in which the deterioration of the overall performance capacity is rapid and the successive testing sessions have to be spaced closely. Five alternative forms of the test are available (*63*). The Ohio State University Psychological Test is another power test of intelligence that has been used because it has several alternative forms (*21*). It stresses verbal ability and consists of synonyms-antonyms, verbal analogies, and paragraph interpretation.

A good example of the rigorously timed speed tests is the Psychological Examination for College Freshmen, prepared under the auspices of the American Council on Education. New forms have been issued annually and an attempt was made to make the successive editions equivalent by the selection of items of known degree of difficulty (*65*). For several years the test consisted of 6 parts, separated into 2 groups, which provide an estimate of the ability to think in quantitative and spatial terms (arithmetical reasoning, number series, geometrical figure analogies) and the verbal ability (definitions, synonyms and antonyms, word analogies). The administration of the test takes about an hour, with a total of about 20 minutes allowed for practice problems and about 40 minutes for the actual tests.

As means for repeated testing of intellective performance capacity, a battery of six short tests was assembled at the Laboratory. It was based primarily on the tests developed for the factorial studies of intelligence (*64*). The tests measured the ability to perceive spatial relations, verbal fluency (recall of words beginning with a specified letter), perceptual speed, memory (strength of word-number associations), number facility (multiplication), and inductive reasoning. The time allotments for individual tests were short (3 minutes or less) and the whole battery was administered in about ½ hour. The time allotments, principles of constructing a large number of alternative forms, the reliabilities of the tests and the relationships to other intellective tests have been described (*20*). The subjects stabilized their work methods and, as a rule, their performance was brought up to a plateau before they were placed on an experimental diet.

Several tests have been used for the study of learning. These have been mostly very brief tests (30 seconds to 2 minutes), involving simple mental operations, administered in 12 to 15 successive sessions given on the same or several consecutive days (*21*). Two of these tests were used in the study on the effect of restricted intakes of B-complex vitamins on intellective functions because they appeared potentially useful in the light of the results obtained by Harrell (*24*, *25*) in studying the effect of added thiamine. In addition to these short tests, in the partial restriction period, the same form of the American Council on Education Test (*65*) was administered on five occasions. Over a period of 7 months the absolute practice gains in the restricted and supplemented group were almost identical. Also, the data on the practice gains in the Repeatable Test Battery during the period of partial restriction were utilized as criteria of learning (*21*). No evidence was obtained that a prolonged restriction of the intake of B-complex vitamins (0.19 mg. thiamine, 0.29 mg. riboflavin, 3.7 mg. niacin) affected learning ability in adult young men.

Comments

The principal methods for studying performance in relation to nutrition have been reviewed. The results of the studies which were cited were of secondary interest and were considered only as illustrative material. A comprehensive review of the older literature on physical performance in relation to diet was prepared by Keys (*33*, *44*), and the review was brought up to date by Simonson (*60*). The problems of industrial nutrition were treated systematically by Pyke (*58*). One of the striking features brought out by this textbook is the extreme paucity of data based on controlled investigations on the effects of diet on industrial output—a fact noted earlier by Simonson (*59*). In this country a limited amount of work has been done on some aspects of industrial nutrition, such as the effectiveness of vitamin supplements ((*3*), with several indices of "industrial morale" rather than output used as criteria), and the frequency of meals (*46*). Literature on meal habits and their effects on performance was reviewed by Hutchinson (*28*). The German data on nutrition and industrial performance were recently summarized by Kraut (*37*); *Nutrition Reviews* (*52*, *53*); and Lehmann (*39*).

In the field of intellective performance examined by standardized psychometric tools, the effects of vitamins of the B complex, especially thiamine (*47*, *50*, *51*, *54*) and of glutamic acid supplementation or restriction, have been studied most intensively. The literature on the latter point is especially large (*48*, *49*) and covers both human and animal experiments.

In the Laboratory of Physiological Hygiene performance aspects of man's fitness were examined with reference to the vitamin content of the diet (*36*) and prolonged caloric restriction (*35*), taking into account gross locomotion, the psychomotor tests of strength, speed, and coordination, as well as intellective and sensory functions and personality. The investigations of Simonson and Enzer (*62*), the work of Keeton and his associates (*31*) on cold tolerance, the Iowa thiamine studies (*66*), and the study on protein requirements carried out at the University of Illinois (*4*) may serve as examples of a fairly broad coverage of different aspects of performance.

The relationships between physiological criteria of performance capacity and the work performance demand further study. In Montoye's investigation (*45*) the Harvard Step Test was used as a physiological fitness index. Three criteria of performance were used: the number of sit-ups, time of a half-mile run, and endurance in pedaling a bicycle ergometer against resistance at the speed of 20 miles per hour. The coefficients of correlation between the step-test scores and the performance criteria were statistically significant for the first two variables but were too low to be useful for predictive purposes (r's of 0.286, -0.232, and 0.091; $N=50$).

Summary and Conclusions

Laboratory studies on nutrition and performance have been concerned primarily with gross physical performance, as it is represented by different types of locomotion, with particular reference to the resulting changes in cardio-respiratory functions and blood chemistry. At the same time, analysis of the occupational demands in the Armed Forces and in industry indicate the importance of manipulative speed and coordination, sensory perception and mental functions which were examined much less thoroughly in reference to diet. This has been due, in part, to the fact that the laboratory tests of sensory functions and intelligence, at least in their classical analytical form, indicated surprising resistance to nutritional stresses while the psychomotor functions were shown to be more susceptible to deterioration.

The potential usefulness of miniature work situations in the study of nutritional stresses remains to be examined. One definite advantage of this research tool is that the work can be continued for a matter of hours while the usual laboratory tests of motor (as well as of sensory and, to a lesser extent, of intellective) functions are short, rarely exceeding 5 minutes. The problem of alertness and its relationship to food intake may also deserve attention (*10*).

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Physiological Alterations in Organic Functions

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It has proved unnecessary for me to discuss at any length two considerations which are important to my topic. The first is adequate statistical methods in the evaluation of data obtained in studies on man. Dr. Crampton and others covered this point yesterday. Today, Dr. Brožek and others have covered the other—the necessity to study function. My presentation will present a philosophy, a way of thinking, developed by my colleagues and me since 1940 during studies on low-calorie rations (*2*).

To illustrate a specific case, three figures are shown of a group of able-bodied, excellent Canadian troops studied by Dr. Kark in 1944 (*3*). Figure 1 shows the group before the field test started. Figure 2 shows the remnants of them 72 hours later after a period of low-calorie, high-fat diet and exposure to extreme cold and hard work. Why had they been taken to the hospital? Figure 3 shows the reason. They were completely "beat." We knew what was wrong with them in a general way: Caloric deficiency associated with ketosis. But we did not know what their weakest links were, what actually broke down. Was it the central nervous system; was it the liver; was it their muscles? In 1944 techniques for diagnosing organ function in field studies were not very numerous or very good. We believe that now it is possible to obtain much important information—on organ function, and that such studies are obligatory in modern field tests.

We should like to present now some aspects of a study completed last year at the University of Illinois through the efforts of Captain F. Sargent, II, and a large staff under an Air Force contract (*6*). The figures which follow will not be used to explain this study so much as they will be used to describe the scientific method which we employed. We feel that emphasis in this problem of survival rations must be placed on the assessment of organ function and the function of the body as a whole in a statistically valid manner. The criteria which we suggest for validity are that in arriving at a decision concerning the relative merits of nutrient combinations, any data should be predictive of potential deterioration, should discriminate among the several variables under consideration, and should be interpretable in terms of current clinical thought.

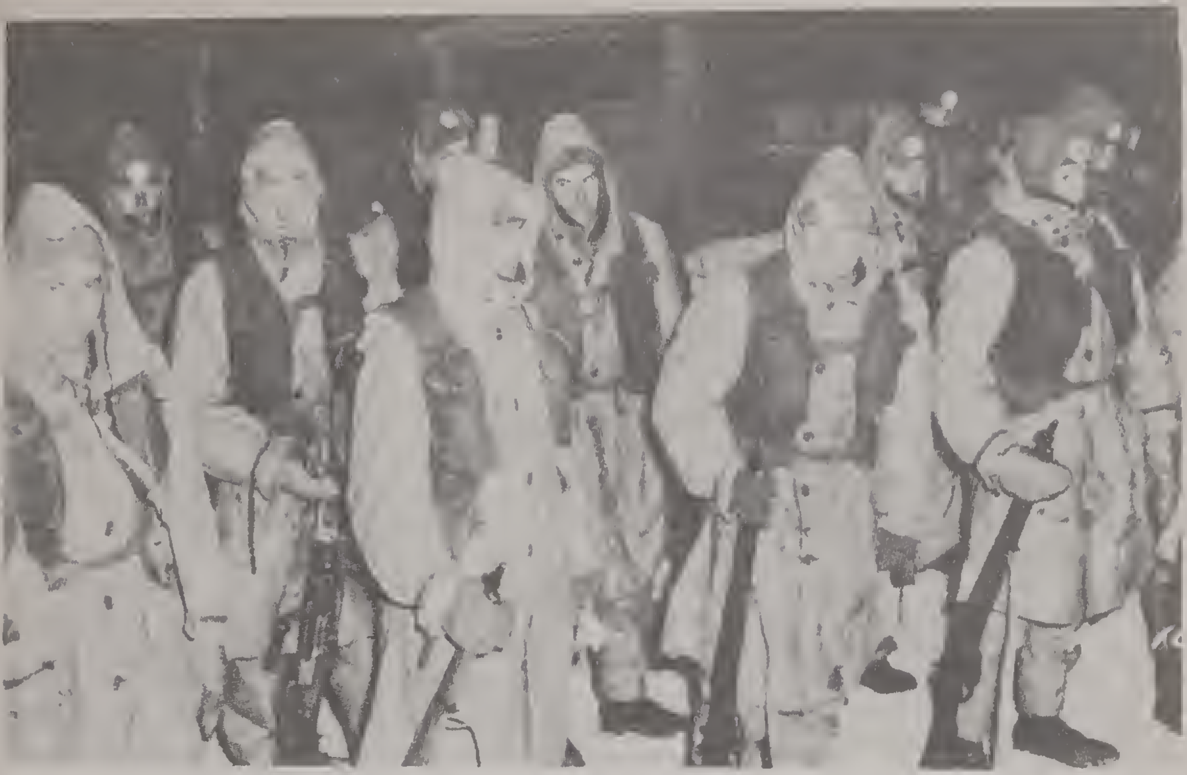

FIGURE 1.—Group of Canadian troops before field test started.

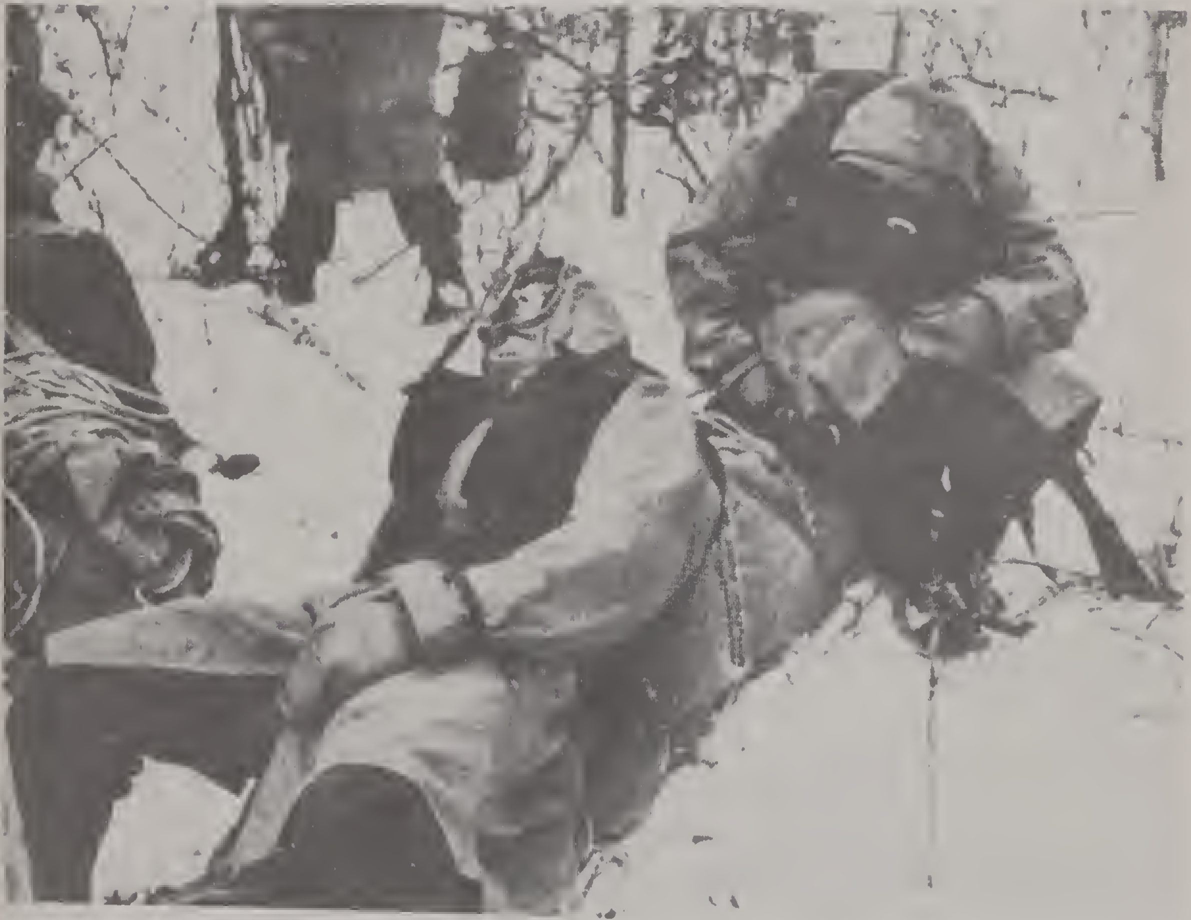

FIGURE 2.—The remnants of the group, 72 hours later, after a period of low-calorie, high-fat diet and exposure to cold and hard work.

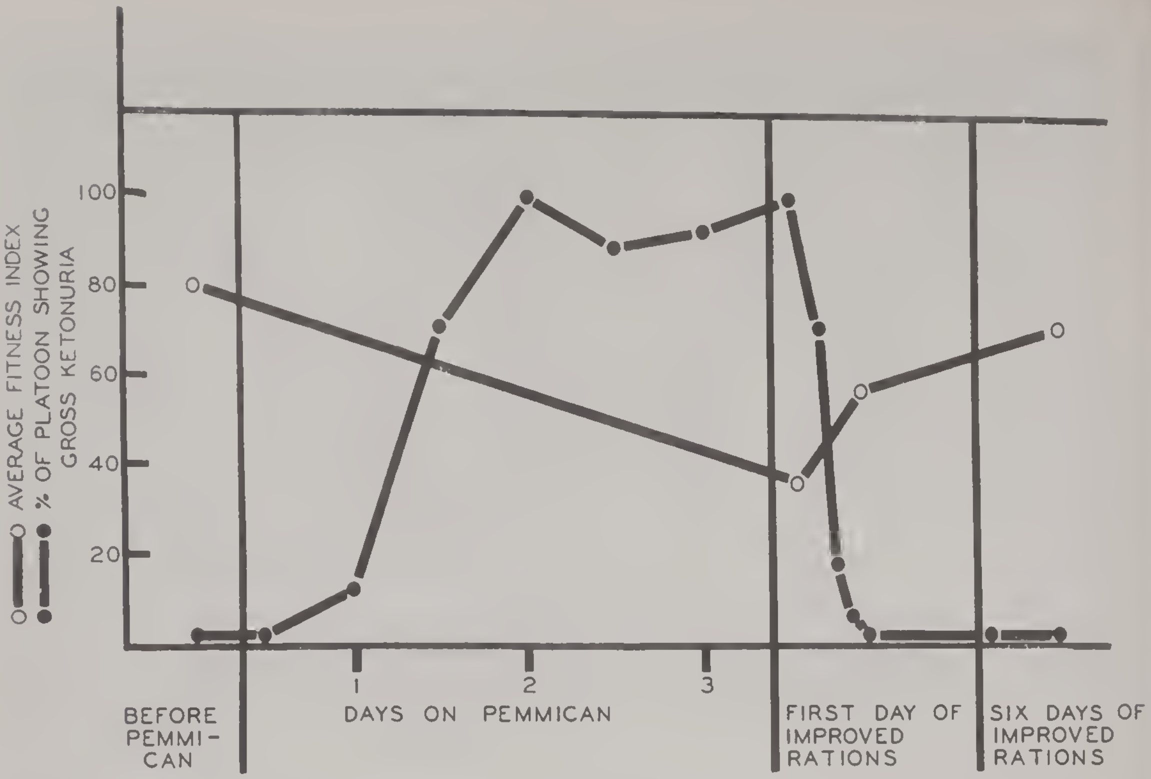

Figure 3.—Physical deterioration and ketonuria of platoon eating pemmican.

For 5 months student volunteers were kept under continuous metabolic observation during alternating periods of adequate diet followed by 2-week periods of restricted diet. I shall not present the details of timetable but say merely that continuous collections of urine and feces were made for 147 days and function studies were conducted weekly. Figure 4 shows the categorization of observation that Dr. Sargent made. You will see in the 10 major blocks the categories: clinical, dietetic, metabolic balance, body composition, clinical pathology, liver function, endocrines, nervous system, kidney function, and gastrointestinal function, each with the individual tests or measurements that were pertinent in the major categories. Much of the information was obtained in what we called the 2-hour test, figure 5. In 120 minutes as shown in the left column, large numbers of observations were made. As shown in the two right-hand columns the data were collected by analysis of the specimens collected during the 2-hour test.

We were interested in studying the interaction of many variables, chiefly water, caloric level and distribution of calories. Figure 6 shows the actual combinations which were employed, and I draw your attention particularly to the right-hand column giving the code symbols for the combinations. These codes will appear in all of the subsequent figures. You will see that there were 20 nutrient combinations covering the variables: limited water, unlimited water; 0, 1,000, 2,000, and 3,000 calories per man per day; and caloric distribution ranging from pure carbohydrate through high protein, high fat, or both, and including a very important mixture which we term

CLINICAL	DIETETIC	METABOLIC BALANCE	BODY COMPOSITION	CLINICAL PATHOLOGY
A. Physical Exams B. Histories C. Cardiovascular 1. B.P. 2. Pulse 3. Circulation time 4. Exercise 5. EKG D Neurological	A. Intake 1. Gross 2. Weighback B. Menus 1. Planning 2. Acceptability a. Psychological	A. Energy B. Water C. Nitrogen D. Na, Ca, K E. Cl, P F. Acid-Base G. Fat Absorption	A. Weight B. Fat, LBM C. Water 1. Antipyrine 2. D_2O 3. Thiosulfate D. Photographs E. Albright Calculations	A. Hematology B. Urinalysis C. Fecal Studies D. Blood Chemistry E. Urine Chemistry F. Blood Enzymes
LIVER FUNCTION	ENDOCRINES	NERVOUS SYSTEM	KIDNEY FUNCTION	G.I. FUNCTION
A. Cholinesterase B. Cephalin Floc. C. Urobilinogen D. Cholesterol E. Blood Sugar F. Bilirubin	A. 17-KS B. Eosinophils C. Resting MR D. Blood Sugar E. Serum Na, K, Ca, P F.. Cholesterol G. Water Diuresis H. Blood Adrenalin I. Blood Enzymes	A. Central 1. EEG 2. Reflex time 3. Reaction time B. Autonomic 1. Pupil size 2. Blood adrenaline 3. EKG, BP, T C. Psyche 1. Biological time 2. Psychological 3. Diary 4. Progress notes	A. Urinalysis B. Addis Count C. Creatinine Clearance D. Ammonia E. Osmotic Clearance F. Blood Urea G. Urine Concentration and Dilution	A. Fecal Weight B. Fecal Fat C. Occult Blood D. Formed Elements E. Clinical

FIGURE 4.—Observations—survival ration study.

PROCEDURE	ANALYSES AND CALCULATIONS	
1. Drink 100-200 ml. of water. 2. Void into 24-hour spec. bottle; note time. 3. Weigh on Sanctorius balance; note time. 4. Recline 30 minutes. 5. Measure oral temp., pulse, B.P., skinfold thickness, passage of time, last 10 min. 6. Ten-minute collection of expired air in Tissot gasometer. 7. Measure W.B.T. and D.B.T. 8. Weigh on Sanctorius balance; note time. 9. Venipuncture; note time. (Lapsed time, 60 min.) 10. Mix expired air, record vol., temp., and barometric pressure; collect sample in 50 ml. oiled syringe. 11. Void at c. 120 min. into special specimen bottle; note time. 12. Measure urinary vol.; save 25 ml. aliquot in special spec. bottle.	A. LIVER FUNCTION 1. Two-hr. urobilinogen 2. Cephalin floc. B. RENAL FUNCTION 1. Min. urine vol. 2. Addis count 3. Creatinine clearance 4. Free NH_3 C. CARDIOVASC. FUNCTION 1. B.P. 2. Pulse D. PSYCHIC FUNCTION 1. Passage of time E. BODY COMPOSITION 1. % Body fat 2. Lean body mass F. METABOLISM 1. O_2 consumption 2. R.Q. 3. Pulmonary vent. 4. Insensible H_2O loss.	G. HEMATOLOGY 1. R.B.C. 2. W.B.C. 3. Differential 4. Eosinophil count 5. Hemoglobin 6. Hematocrit 7. Sed. rate 8. Platelet count

FIGURE 5.—Protocol of organic function—resting metabolism test.

EXPERIMENTAL RATIONS AND OTHER FOODS USED	CALORIC INTAKE	% DISTRIBUTION OF CALORIES	SYMBOLS USED IN TABLES AND FIGURES
Pre-Period: 5-in-1	c. 3600	14% P, 50% CHO, 36% F	PRE, Day 0
Positive Control: 5-in-1	3000	14% P, 46% CHO, 40% F	N 3000
Recovery: 5-in-1 plus Ice Cream, Fresh Bread, Oleomargarine, Orange juice.	c.4400	13% P, 48% CHO, 40% F	REC
Negative Control: Starvation	0		ST. 0
Spice Drops, Starch Jelly Bar, Hard Candy, Jam, Sugar, Crackers.	1000 and 2000	0% P, 100% CHO, 0% F	0/100/0 1000 0/100/0 2000
Chocolate Bar, Crackers, Oleomargarine.	1000 and 2000	2% P, 20% CHO, 78% F	2/20/78 1000 2/20/78 2000
Meat Bar and Cereal, Biscuit or Crackers, Jelly, Pineapple, Sauce, Hard Candy.	1000 and 2000	15% P, 52% CHO, 33% F	15/52/33 1000 15/52/33 2000
Meat Bar	1000 and 2000	30% P, 0% CHO, 70% F	30/0/70 1000 30/0/70 2000
Water Limited; 900 ml./day			L
Water Unlimited: ad libitum			U

FIGURE 6.—Experimental nutrient mixtures.

the normal mixture since its caloric distribution approximates that which most of us eat in our daily life. Figures 7 and 8 show caloric balance during the entire study, and I will not cover this in detail. The left-hand bars show intake. The right-hand bars show caloric output. In the two categories light activity and moderate activity, when the left-hand bar is higher than the right, the subject was in positive balance. Note the striking difference between starvation and the 3,000 calorie normal mixture. Figures 9 and 10 show water balance. Intake included fluid and water in the food; output included loss in the urine, insensible water loss through the lungs and skin; and the miscellaneous item included water in the feces and in the blood which was withdrawn weekly. In general, water balance was

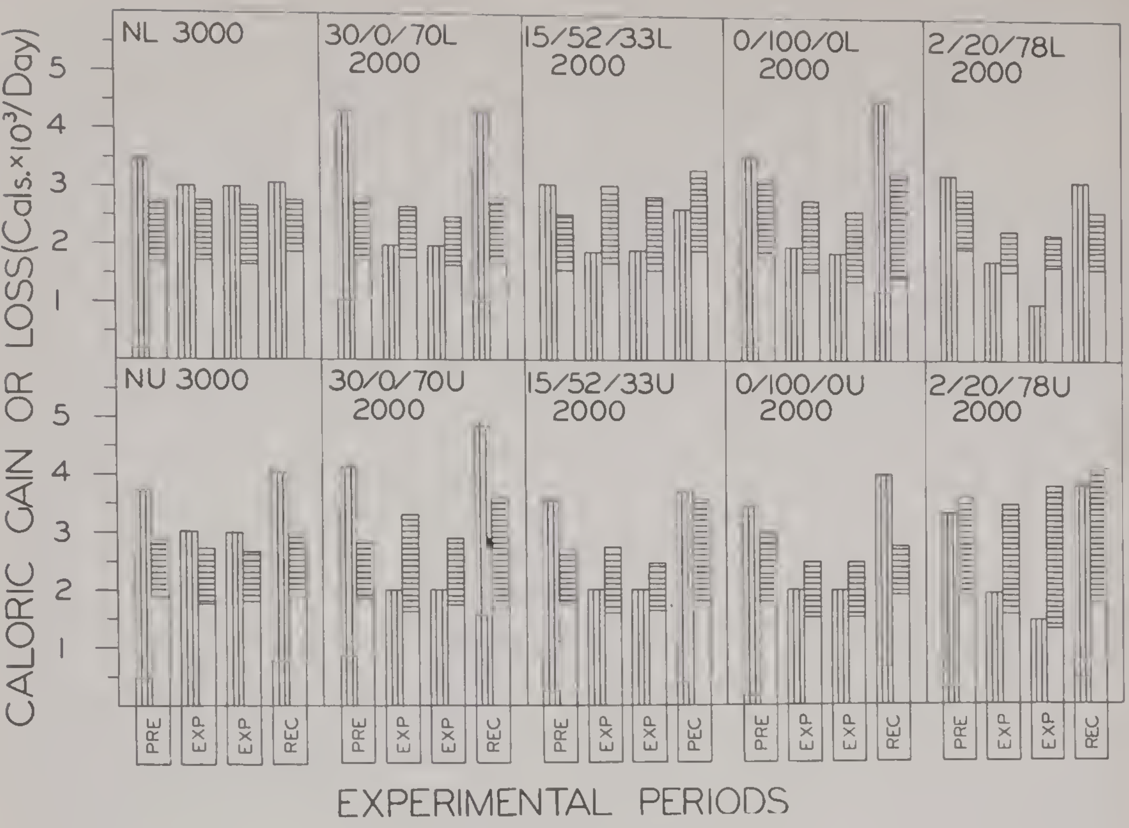

FIGURE 7.—Calorie balance—I.

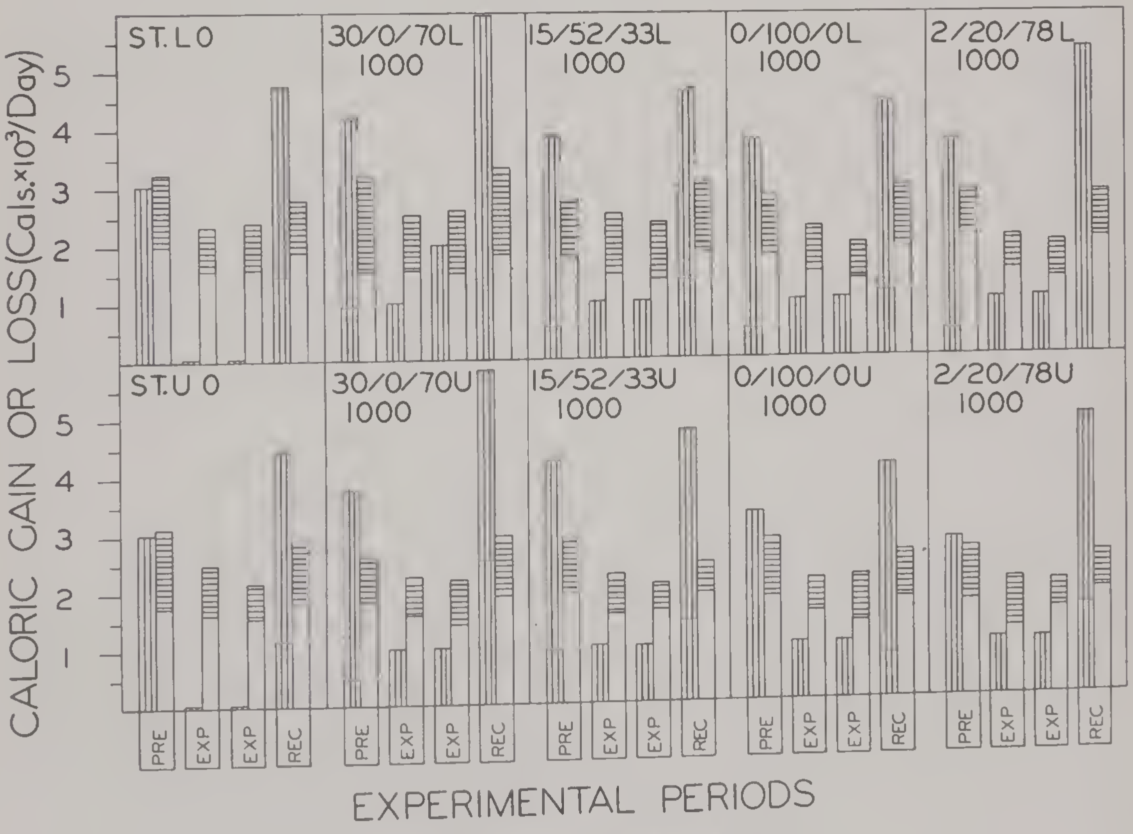

FIGURE 8.—Calorie balance—II.

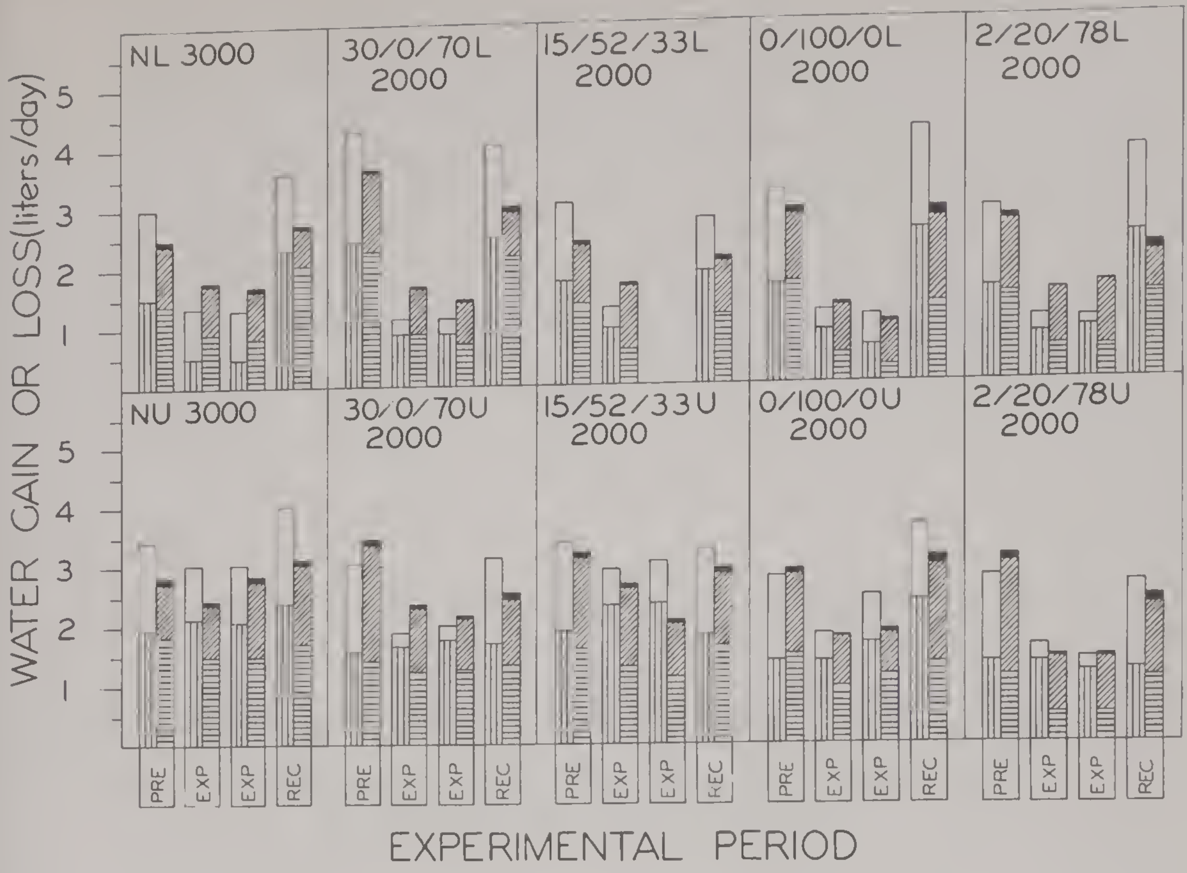

FIGURE 9.—Water balance—I.

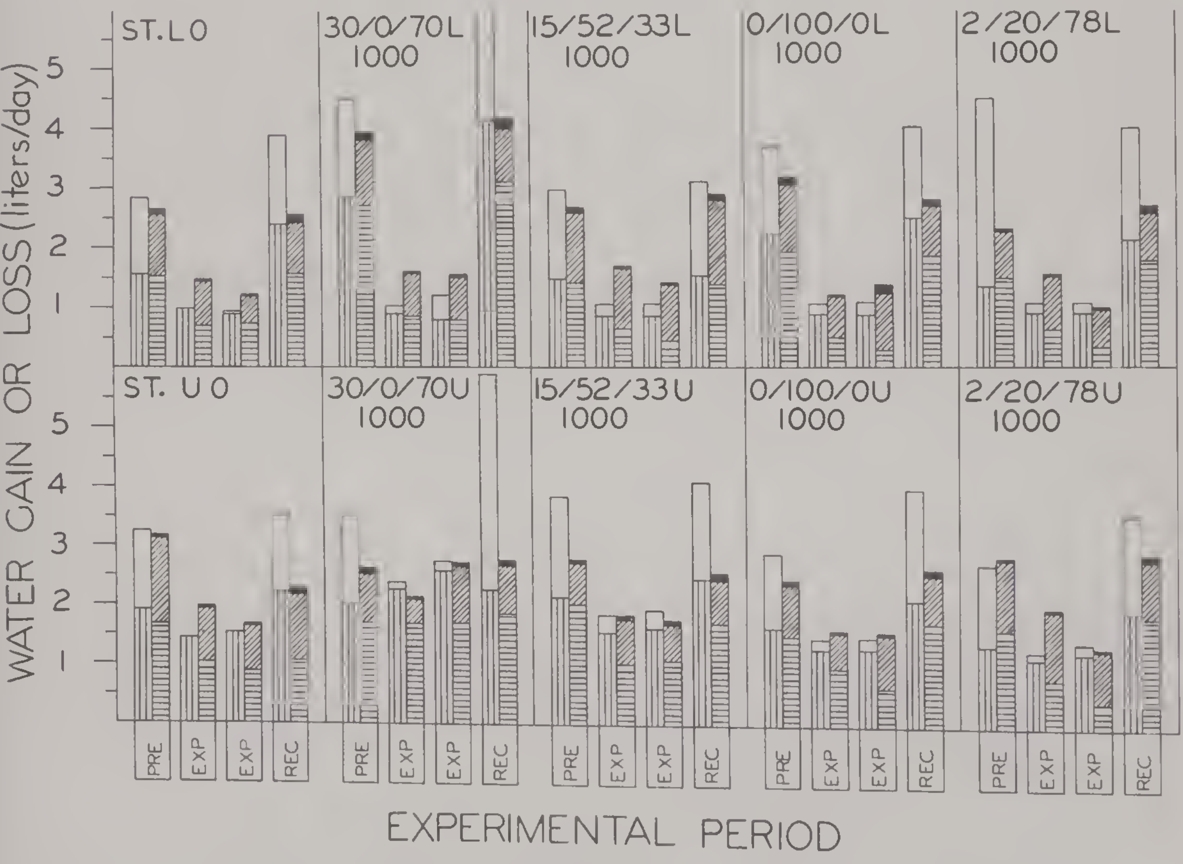

FIGURE 10.—Water balance—II.

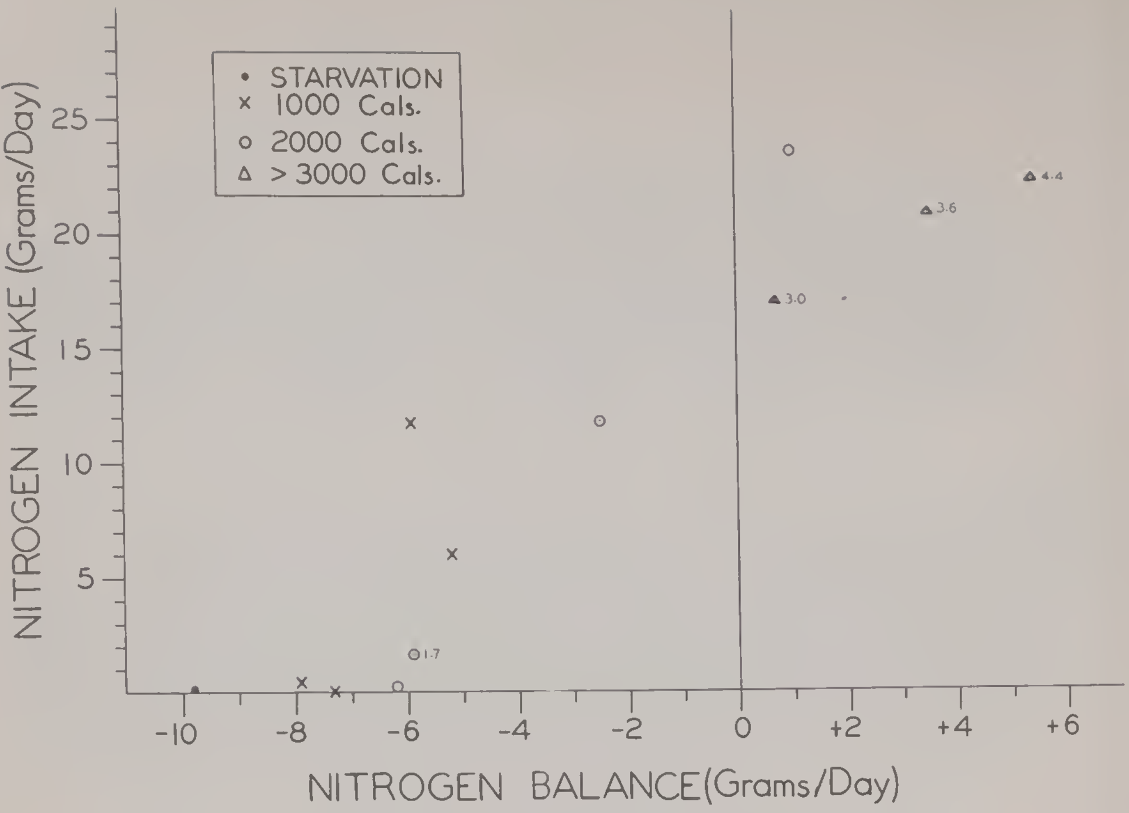

FIGURE 11.—Nitrogen balance vs. nitrogen intake.

well maintained during unlimited regimens and was invariably negative with water deprivation. Figure 11 summarizes the averages of all the data on nitrogen balance for all subjects for experimental periods, as well as for recovery periods. The ordinate is intake as measured by analysis and the abscissa is nitrogen balance as calculated from the analysis of all excreta including blood loss and cutaneous loss. You will see that we have confirmed the classic view that increase of nitrogen intake usually improves nitrogen balance, and also the finding that increasing the caloric intake during periods of negative nitrogen balance improves the balance slightly (*5*). Note, however, that isocaloric addition of nitrogen at caloric levels of 1,000 had little if any effect on nitrogen balance. Is it possible that there is a critical caloric intake, below which isocaloric addition of nitrogen is ineffective in diminishing a negative nitrogen balance (*8*)?

Figure 12 shows one measure of adrenocortical function. Note that limitation of water had little effect on this excretion but that an experimental dietary stress invariably led to a decrease in excretion of 17-ketosteroids. Starvation led to a decrease of 50 percent, the normal 3,000 calorie mixture to a decrease of only 10 percent. We have adopted Sayers' interpretation that these experimental periods require an increased utilization of adrenal cortical hormones by the tissues, resulting in a decreased excretion of steroid metabolic products (*7*).

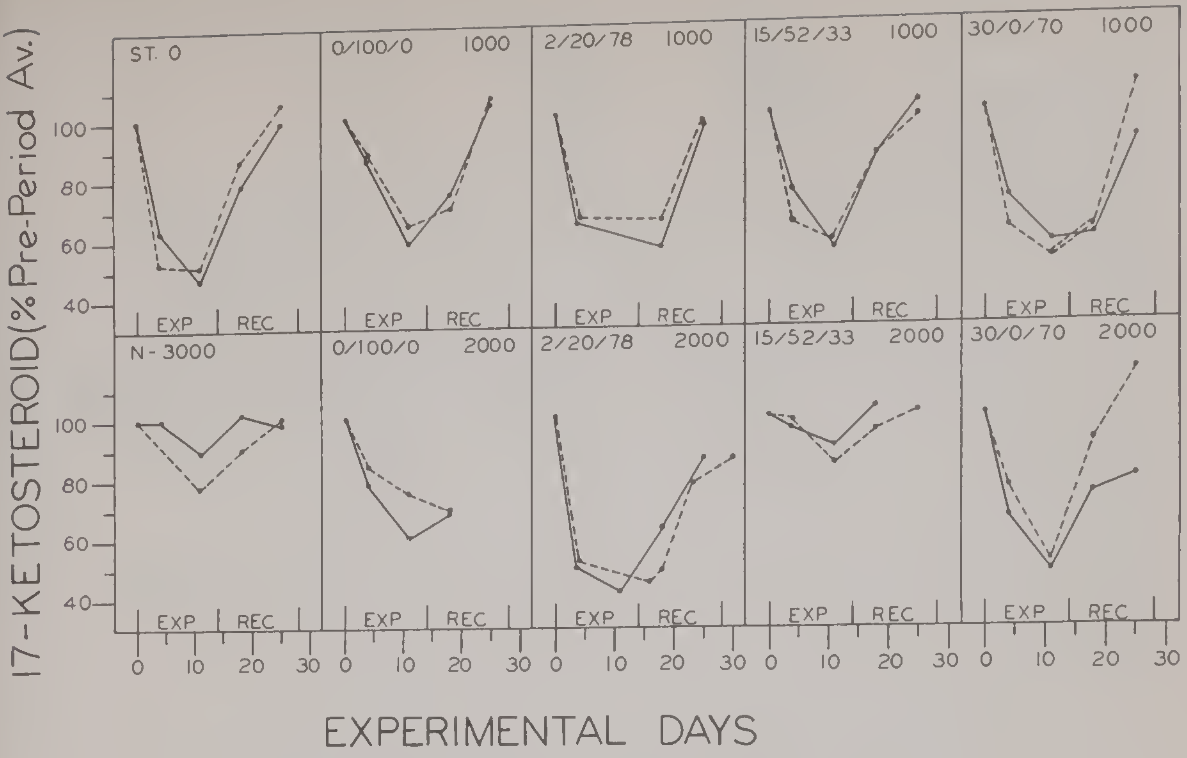

FIGURE 12.—Urinary 17-ketosteroid excretion.

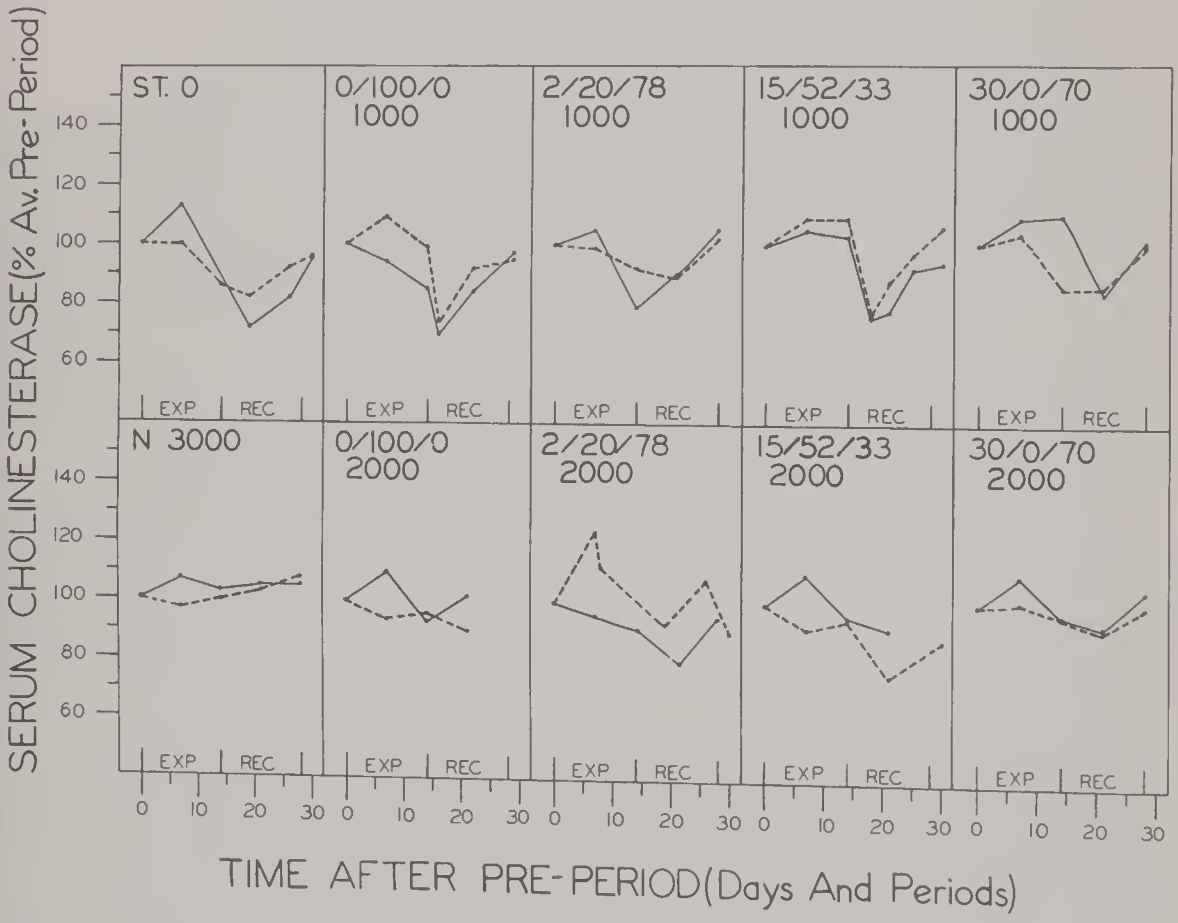

FIGURE 13.—Liver function—serum cholinesterase.

Figure 13 shows the most sensitive test of liver function that we used: serum cholinesterase. Note the decrease in starvation and the lack of change during the 3,000 calorie normal regimen.

Figure 14 shows one of the most striking findings that we observed. The erythrocyte sedimentation rate increased during dietary stress

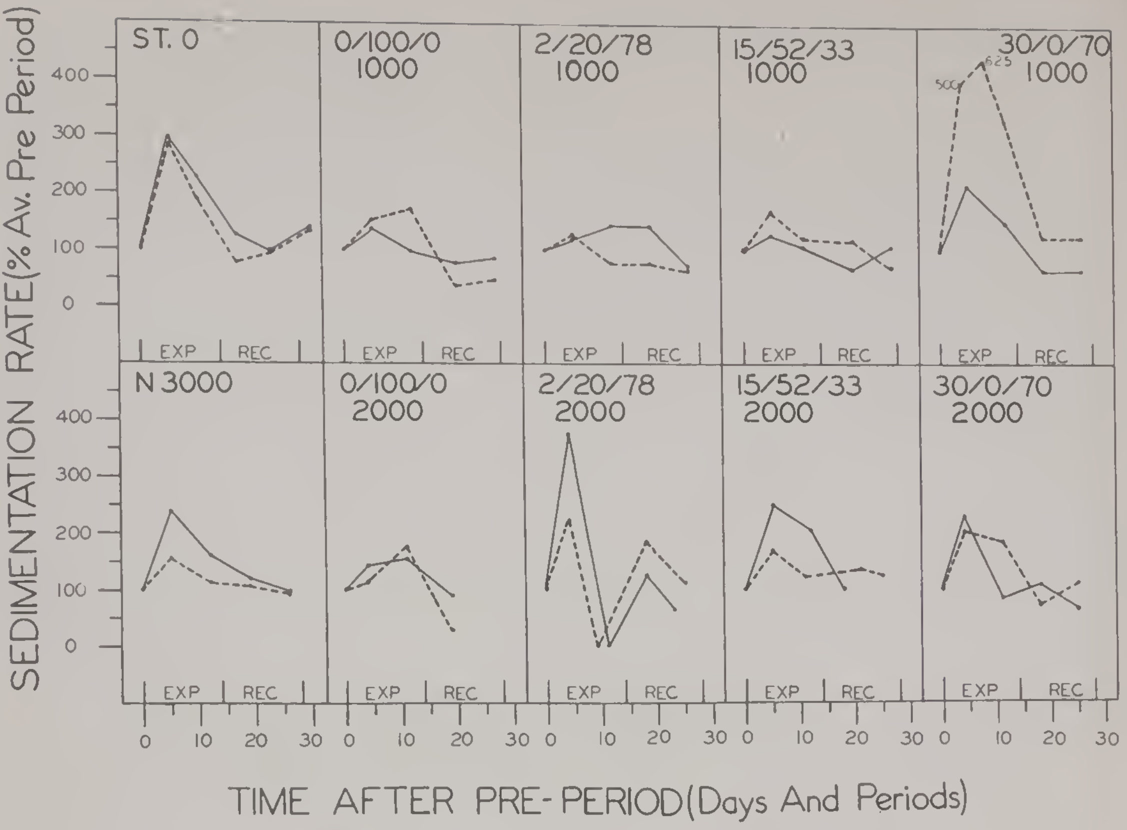

Figure 14.—Hematology—sedimentation rate.

and indeed reached almost pathological levels in two regimens. It was quite high in starvation and moderately elevated in the 3,000 calorie regimen. This clinical test is known to be correlated with: (*a*) the body's response to chronic and acute infection; and (*b*) radiation damage. We hope that it will prove to be useful in nutritional studies. No other hematologic measurement showed consistent changes during our study except the hematocrit, which correlated with dehydration. The erythrocytes, white blood cells, and platelets remained within normal limits throughout the 5 months.

Figure 15 shows the endogenous creatinine clearance during experimental and recovery periods. This measure of glomerular filtration rate proved to be correlated with nutritional stress, showing a large decrease in starvation and a much less noticeable decrease in the 3,000 calorie normal mixture and the 2,000 calorie normal mixture.

Figure 16 is one that I should like to discuss at some length because it represents an important aspect of survival rations, but unfortunately time does not permit a thorough analysis. You will recall that Butler, Gamble, and others have used the obligatory water requirement as a measure of the mineral water requirement in the castaway (*1*). If you will look at the asymptote approached by our subjects' U/S ratios you will see that the minimum urine volume varied with the solute load, being almost a liter in positive control and the meat bar-cereal regimens but only three-tenths of a liter in pure carbohydrate. This asymptote represents the obligatory water requirement of the kidney and shows the kidney working at maximal

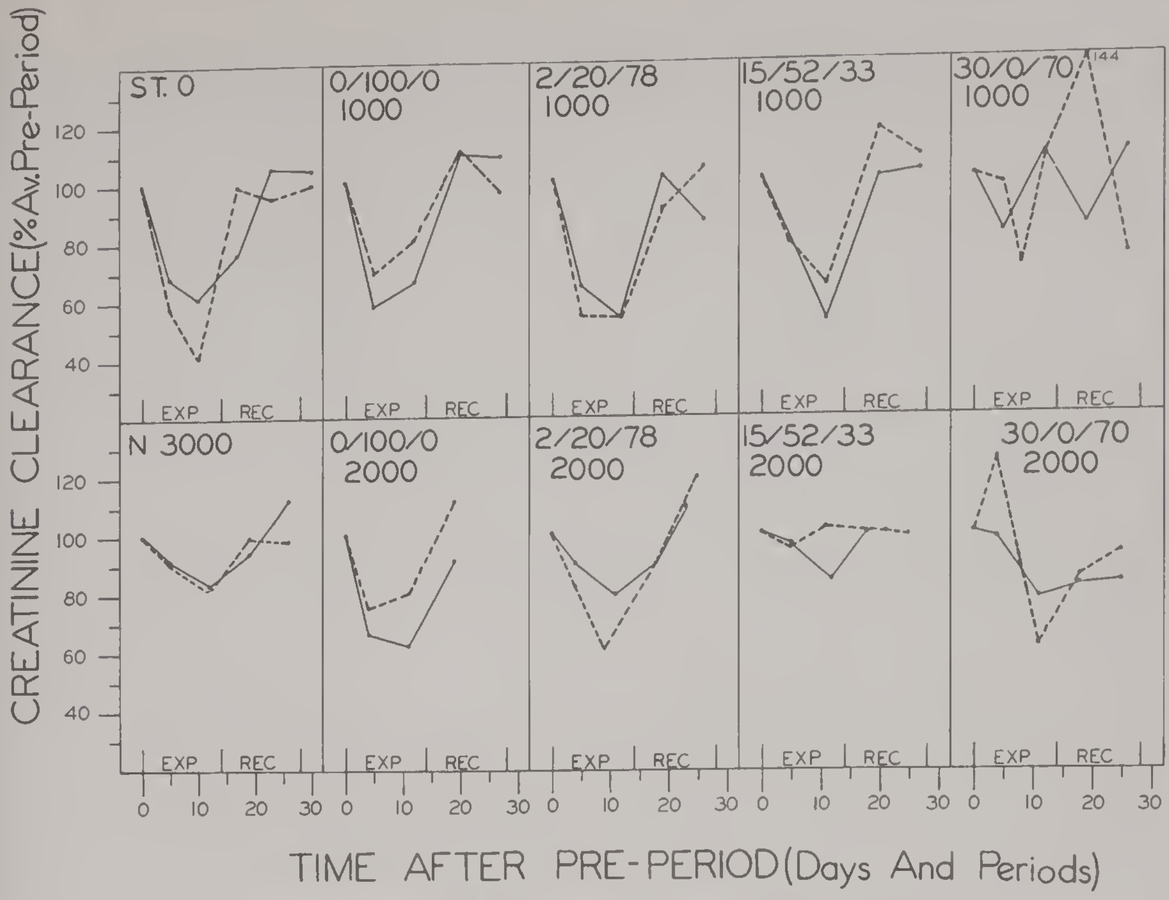

FIGURE 15.—Renal function—"creatinine" clearance

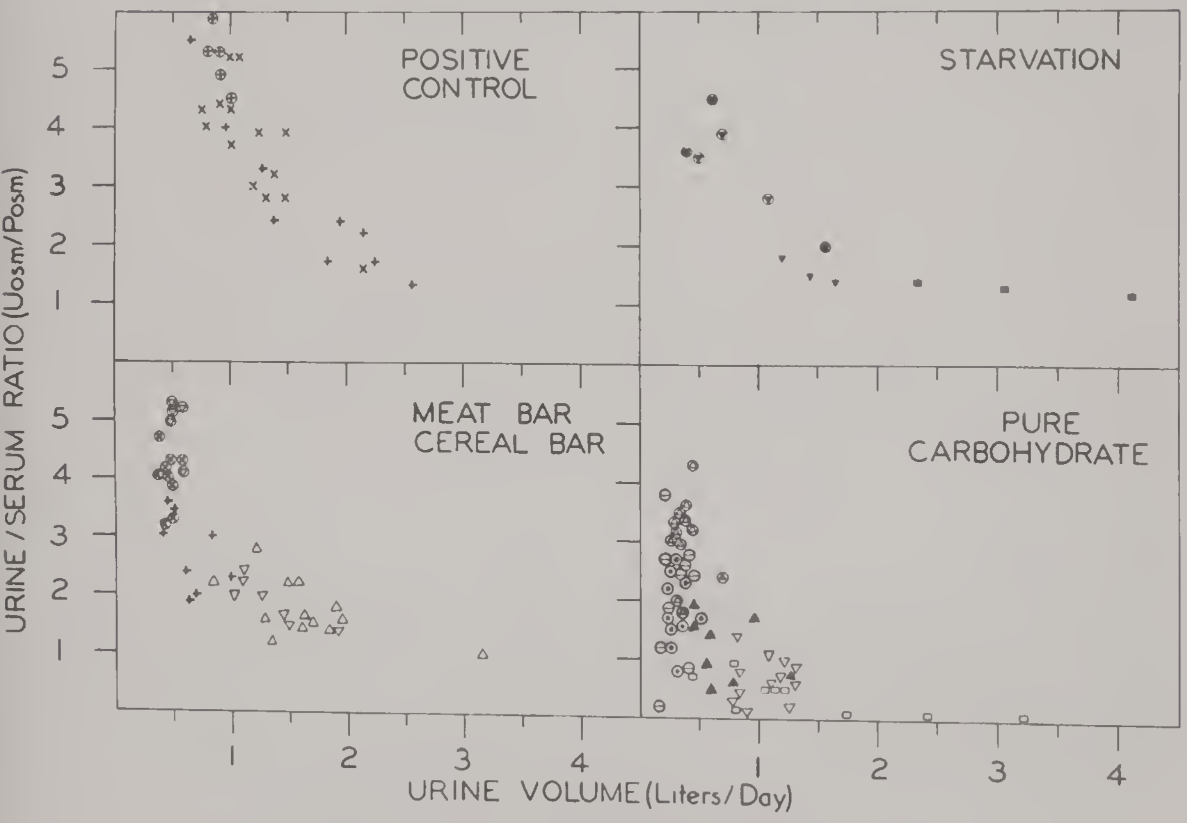

FIGURE 16.—Osmotic ratio vs. solute load.

concentrating capacity. The other asymptote we believe to be equally important if not more so. This is the asymptote approached when urine volume is large. When the U/S ratio reaches one, the kidney is doing minimal osmotic work. You will see that in the regimens of

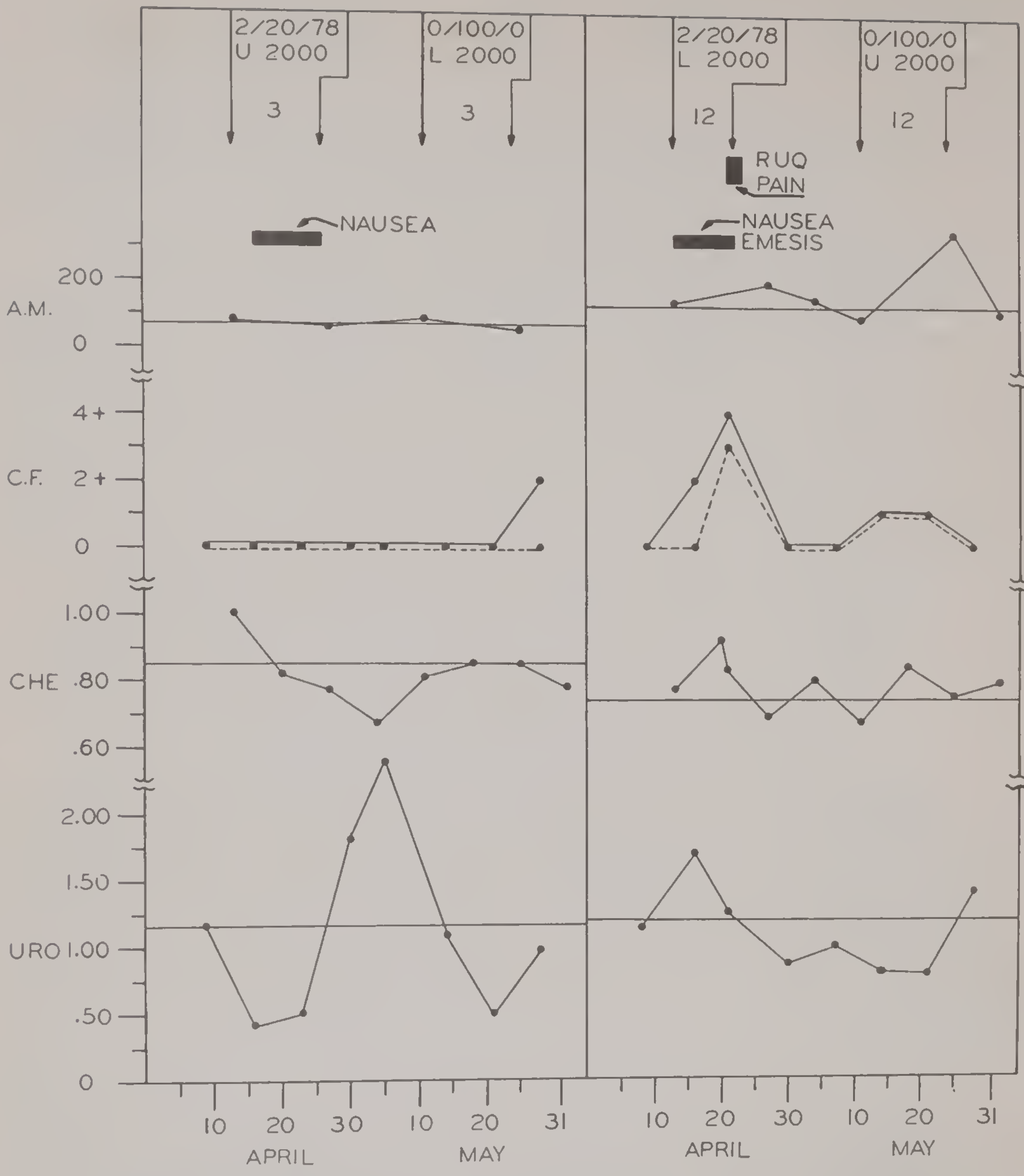

FIGURE 17.—Organic functional reaction—I. (Subjects 3 and 12)

high solute load this U/S ratio of one is approached at urine volumes of as much as three liters per day whereas in the pure carbohydrate regimen it was reached at urine volumes much less than one liter per day. The functional implications of U/S ratio one are of obvious importance if the castaway's renal function is going to remain normal for a prolonged period. In this particular respect, that regimen is best in which the isosmotic point is reached with the smallest intake of water.

So much for the quantitative tests. Clinical considerations also must be given due weight, for sometimes they are paramount. On occasion some subjects became ill—so ill that they had to be removed prematurely from the regimen. Figure 17 summarizes such an incident and its course. All the serious illnesses in our subjects occurred when the subjects were on limited water (900 ml. per day). Subject 12 developed nausea, vomiting, and right upper quadrant pain while subsisting on the high-fat, low-protein ration with limited water. It was necessary to change his regimen at once because our attending physicians felt that he had biliary dyskinesia. Subject 3 on the same regimen, but with unlimited water, did develop nausea but nothing else. Note the increase in serum amylase and cephalin flocculation in subject 12. Other incidents will not be presented at this time.

When all of the data were assembled and anaylzed in relation to the nutrient combinations it proved possible to assign them quantitative values which could be rank-ordered. Among the many measurements made, some did not satisfy our criteria of pertinence (predictive of potential deterioration, discriminative among nutrient combinations, and interpretable in terms of current clinical thought) but 19 different measurements did prove usable. They may be categorized as follows:

(*a*) Nutrient balances—calorie; water; nitrogen; sodium; potassium; phosphorus; acid-base (as measured by urinary execretion of acetone bodies).

(*b*) Body composition—change in body weight; change in body water.

(*c*) Liver function—serum cholinesterase.

(*d*) Kidney function—creatinine clearance; mean volume of daily urine; osmolar clearance as related to the urine/serum osmolar ratio; formed elements in the urine (Addis count).

(*e*) Endocrine function—urinary excretion of neutral 17-ketosteroids (adrenal cortex); blood sugar (pancreas); resting metabolism (thyroid).

(*f*) Hematology—red cell sedimentation rate (a clinical measure of response to some kinds of noxious stimuli).

Many other measurements were made but not used in the final evaluation. Either these measurements showed no difference between nutrient combinations or they did show differences which were not interpretable at present in terms of "survival potential" (*4*). They may be categorized as follows:

(*a*) Nutrient balances—calcium; chloride (same as sodium).

(*b*) Body composition—body fat; lean body mass.

(*c*) Liver function—urobilinogen; cephalin flocculation; serum cholesterol.

(*d*) Kidney function—urinary specific gravity and total solids; serum urea nitrogen; urinary ammonia.

(*e*) Gastrointestinal function—characteristics of feces; fecal fat and fat absorption; qualitative examination of feces.

(*f*) Respiratory function—pulmonary ventilation; respiratory quotient; net oxygen consumption during physical work.

(*g*) Cardiovascular function—pulse rate, blood pressure; circulation time; electrocardiogram.

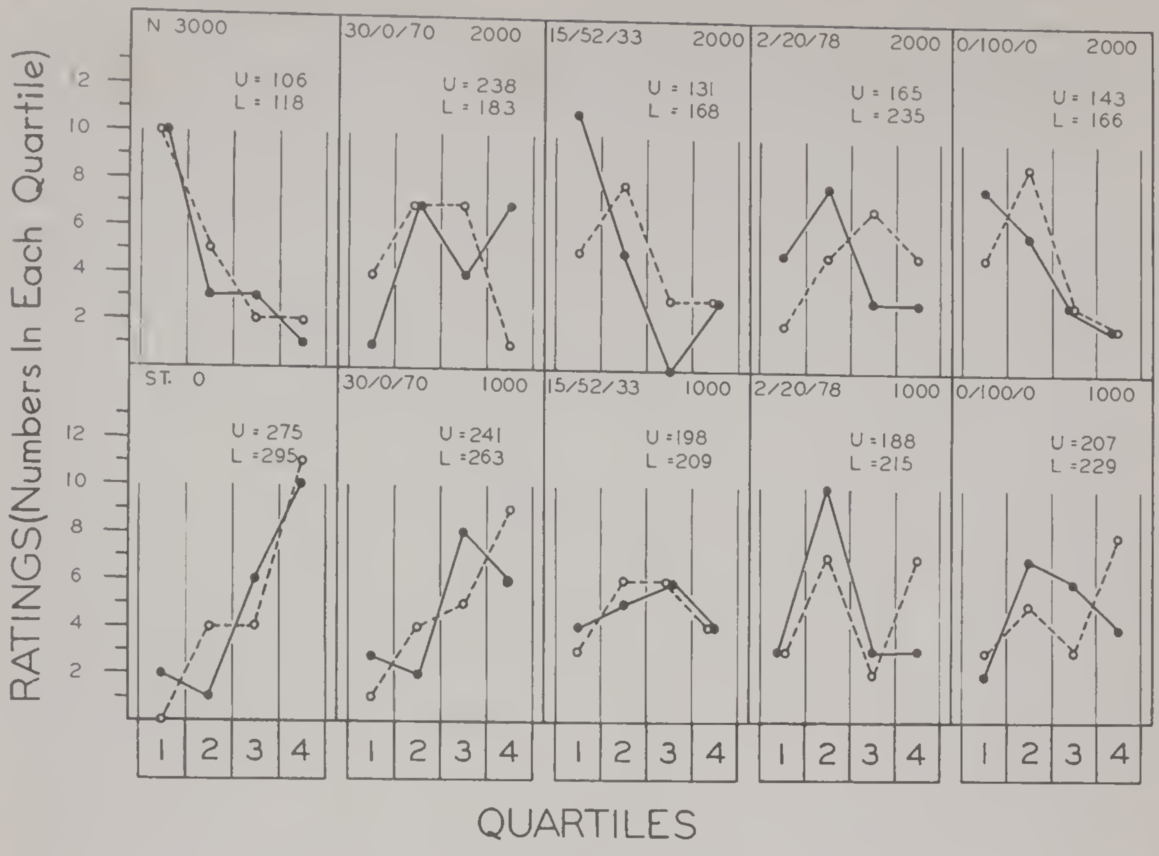

FIGURE 18.—Rank-order: all nutrient combinations.

(*h*) Nervous system—reaction time; reflex time; psychological tests; electroencephalogram.

(*i*) Endocrine function—exocrine function of pancreas; serum calcium and phosphorus; urinary creatine; serum sodium; potassium; and chloride.

(*j*) Hematology—red blood cell count; hemoglobin; hematocrit; total white cell count; direct eosinophil count; thrombocytes; differential leukocyte count; erythrocyte indices; total serum protein.

Figure 18 shows a summary of our quantitative ratings. There were 20 nutrient combinations and 19 descriptive measurements. If a particular combination was first all the time its score would be 19. If it were worst all the time its score would be 380. In examining the figure, note the nutrient combination at the top of each block, the total gross numerical score for the nutrient combinations with unlimited or limited water, and the quartiles in which the nutrient combinations scores fell. The positive control ration (N 3000) proved far the best even when water was limited. Starvation proved the worst even when water was unlimited. Other nutrient combinations fell between these two controls. Clinical judgments were also important in deciding the final ranks. Without going into detail I wish to point out that, all things considered, the "normal mixture" at 2,000 calories was the combination next best to N 3000.

Thus we have applied systematically a philosophy that every effort should be made to measure in quantitative terms the functioning of organs, systems, and the whole body, whenever a nutritional study is conducted on military populations. We feel that many techinques are available at present which are applicable in this kind of clincal investigation.

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Some Aspects of Nutritional Problems of Seriously Wounded Soldiers[1]

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This morning, I should like to discuss briefly some aspects of one of the important nutritional problems encountered during the recent Korean conflict. I have reference to the rapid nutritional deterioration of soldiers with extensive battle wounds, particularly those with serious renal dysfunction. Under these circumstances, open wounds were often described as indolent, with granulation tissue either absent, or when present, soggy and edematous. In a number of these patients, progressive necrosis and suppuration of the wounds was described. Morbidity was prolonged and mortality high.

The following case report is indicative of the complexity of the problem.

Patient No. 33.—This 21-year-old American soldier was injured by a mortar shell. When he reached a Clearing Company 3 hours later, he was in shock and was given whole blood. He reached a Mobile Army Surgical Hospital 5 hours after injury in deep shock; blood pressure, 80/60. The heart sounds were almost inaudible; a cardiac tamponade was suspected and was confirmed by aspiration. Immediate transfusion raised the blood pressure promptly to 130/90 within 15 minutes; he received a total of 300 cc. blood preoperatively and was operated on 7 hours after injury. At operation, he was found to have a hemopericardium, left hemothorax with fractures of ribs, 5–8, contusion of the lower lobe of the left lung, laceration of the left diaphragm, multiple perforations of the stomach, jejunum, and splenic flexure of colon, laceration of the spleen and left kidney, fracture, compound, comminuted, of left humerus, left carpal bones, and left tibia and fibula. The following operative procedures were done: resection of the anterior thirds of the left sixth and seventh ribs with insertion of two thoracotomy tubes; repair and debridement of the left diaphragm; closure of perforations of stomach and jejunum; exteriorization of splenic flexure of colon; splenectomy; removal of foreign body from cortex of left kidney; drainage tubes, left flank; debridement of left chest; irrigation and splinting of the left wrist, humerus, and tibia and fibula. During the operation, he received an additional 3,000 cc. blood. After operation he required 1,500 cc. more blood before his blood was stabilized above shock levels, 7 hours postoperatively, 14 hours after injury.

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[1] The opinions expressed in this paper stem from a trip to Korea by the senior author as a consultant to the AMSGS Surgical Research Team in January and February 1953. The opinions do not necessarily reflect the opinions of the members of the Surgical Research Team at the 46th Mobile Army Surgical Hospital and the Renal Insufficiency Center at the 11th Evacuation Hospital in Korea.

Because of oliguria on the first and second postinjury days, he was transferred to the Renal Insufficiency Center. On admission there, he was in good general condition. Blood pressure, 130/80; pulse, 118; temperature, 100°; respirations, 26. The exteriorized loop of colon was opened to form a double-barrel colostomy. The extremity wounds were infected, and maggots were present in the wound in the left wrist. The thoracotomy tubes were removed when a chest X-ray showed only slight atelectasis at the left base. Because of the hyperkaliemia, an immediate extracorporeal hemodialysis on an artificial kidney of the Kolff type was performed; the K fell from 6.2 to 3.8 mEq/1 plasma and the NPN from 154 to 92 mg. percent. The EKG became normal.

Oliguria continued until the sixth post-injury day. Thereafter, he voided increasing large amounts and by the 12th postinjury day, a peak daily urinary output of 3,000 cc. was reached. Despite the diuresis, the plasma NPN had climbed to 360 mg. percent on the tenth day. He was lethargic, passed tarry stools through the colostomy and would not take fluids or food orally. A repeat hemodialysis was done with a reduction of the plasma NPN from 360 to 54 mg. percent.

During the next day, the tarry stools from the colostomy continued and 1,000 cc. blood was required to maintain his blood pressure. He began to run a fever, up to 103° rectally, and WBC rose to 28,000 with a shift to the left. A chest X-ray was negative save for minimal atelectasis at the left base. Antiobiotic therapy was changed from terramycin to penicillin and streptomycin. Despite this, the fever continued to rise and on the 15th postinjury day he was found to have a large pelvic abscess.

With the onset of the pelvic abscess and the high fever (reaching peaks of 105° daily) he began to deteriorate clinically, becoming weaker and less responsive. Weight loss was marked and he took on a cachectic appearance. The extremity wounds remained in poor condition, with no healing and much purulent drainage. On the 18th postinjury day, a large abscess appeared in the left anterior lower leg, at the site of a previous cut down. He became intermittently delirious. The penicillin-streptomycin therapy was discontinued and terramycin reinstituted.

By the 24th postinjury day, the pelvic abscess seemed smaller and more fluctuant; temperature was 103° and WBC, 19,500. He was weak and disoriented. Feedings through a gastric tube were begun.

The plasma NPN was up to 196 mg. percent despite daily urinary excretions of over 1,500 cc. The wounds, including the areas around the wire sutures of the laparotomy incision, were in extremely poor shape, with little healing evident.

By the 26th day, the plasma NPN had risen to 250 mg. percent and his clinical condition was rapidly deteriorating. It was felt that the continued uremia might be accentuated by the necrotic tissue in the wounds and the massive infection in his peritoneal cavity. Another hemodialysis was done with a drop in plasma NPN from 240 to 99 mg. percent. During the dialysis, his blood pressure fell to below 60 systolic and remained low for an hour despite the use of 2,500 cc. blood. Following dialysis, the blood pressure came up to 100 systolic; the patient seemed more alert and oriented for about 24 hours.

Two days later, his blood pressure again fell; he became much weaker and became almost nonresponsive. The plasma NPN was up to 203 mg. percent. The urinary volume, which had dropped abruptly to 200 cc. on the 26th day, rose to 1,300 cc./day. His temperature was 102° and WBC, 26,000. His blood pressure continued to fall and was unobtainable the next day. His respirations became gasping and no cardiac sounds could be heard after a shift in his position on the Stryker frame. Intracardiac adrenalin gave a prompt and vigorous return of cardiac action. He was then given norepinephrine intraveneously by slow drip. With this, his blood pressure and pulse were maintained at satisfactory levels, but he remained comatose, non-responsive, and expired on the 30th day after injury.

In the patients with extensive wounds and renal failure, hyperkaliemia, uremia, wound and pulmonary infections, and severe malnutrition were important complications. Weight losses of 20 to 30 pounds in 2 to 4 weeks were frequent, and losses up to 45 pounds in the same period of time were not uncommon. Malnutrition after injury was not limited, however, to those patients with battle wounds and concomitant renal failure but occurred in many seriously injured patients without renal dysfunction, the more severe the trauma, the greater the nutritional disturbance.

Several factors are involved in the development of malnutrition following injury. Derangements in water, electrolyte, vitamin, fat, carbohydrate, and protein metabolism occur. Characteristically, an injured individual excretes excessive amounts of nonprotein nitrogen in his urine for 3 to 7 weeks following injury. During this time he is in negative nitrogen balance and is gradually losing body protein. The intensity and duration of this period of protein depletion depends on a number of factors, among which are the extent of the injury and the state of the individual at the time of injury. However, we still are not entirely clear as to what the basic mechanism is for the metabolic derangements after injury. There are a number of factors involved, including autolysis and absorption of tissue breakdown products, imbalance of endocrine activity, inactivity and reduced dietary intake. The possibility that a disturbance in the metabolism of one or more of the essential amino acids is one of the important factors in the pathogenesis of the increased catabolism following injury was suggested a number of years ago by various investigators. However, there have been no conclusive data in this regard. Plasma amino acids have been little studied in the past for lack of appropriate methods. In the past few years, with the advent of ion exchange chromatography, it has become possible to separate quantitatively each of 19 amino acids, and to study their quantitative relations to the other plasma NPN components. We have been investigating these problems in battle injuries with and without associated renal failure. Samples of plasma were collected by members of the Surgical Research Team at the 46th MASH and at the 11th Evacuation Hospital (the Renal Insufficiency Center). These samples were immediately frozen and shipped in the frozen state to the Army Medical Service Graduate School.

Figure 1 shows a representative separation of the plasma amino acids obtained by the ion exchange chromatographic technique. The peak labeled serine plus glutamine plus asparagine can be separated by isolation, hydrolysis, and subsequent rechromatographing; the result of this procedure is shown in the upper right section of figure 1. The level of each plasma amino acid in normal adults is fixed near a given value, and in health deviates from this level only slightly.

It had been previously thought that trauma of various types is followed by a general rise in plasma amino acids. We have found that such is not the case. For example, in patients with battle wounds and renal dysfunction, the total nitrogen of the free amino acids remained very close to normal, in spite of extremely high NPN's—some higher than 400 mg. percent. (See figure 2.) Examination of the individual amino acids reveals that each of the plasma amino acids reacts to injury of the organism in its own fashion. Similarly, the total free amino nitrogen concentration was little affected by extracorporeal dialysis for 6 hours by an artificial kidney of the Kolff type.

We studied variations in the plasma amino acid levels and the other NPN components in 5 critically wounded young soldiers. Four of these men died. Shock and renal failure were present in all in varying degrees. In four, the renal failure was persistent, with eventual plasma urea nitrogen levels up to 400 mg. percent. Extracorporeal dialysis by a Kolff type artificial kidney was performed in three for the purpose of reducing hyperpotassemia or relieving uremia. Temporary biochemical and/or clinical improvement followed the dialysis, but eventually, all died.

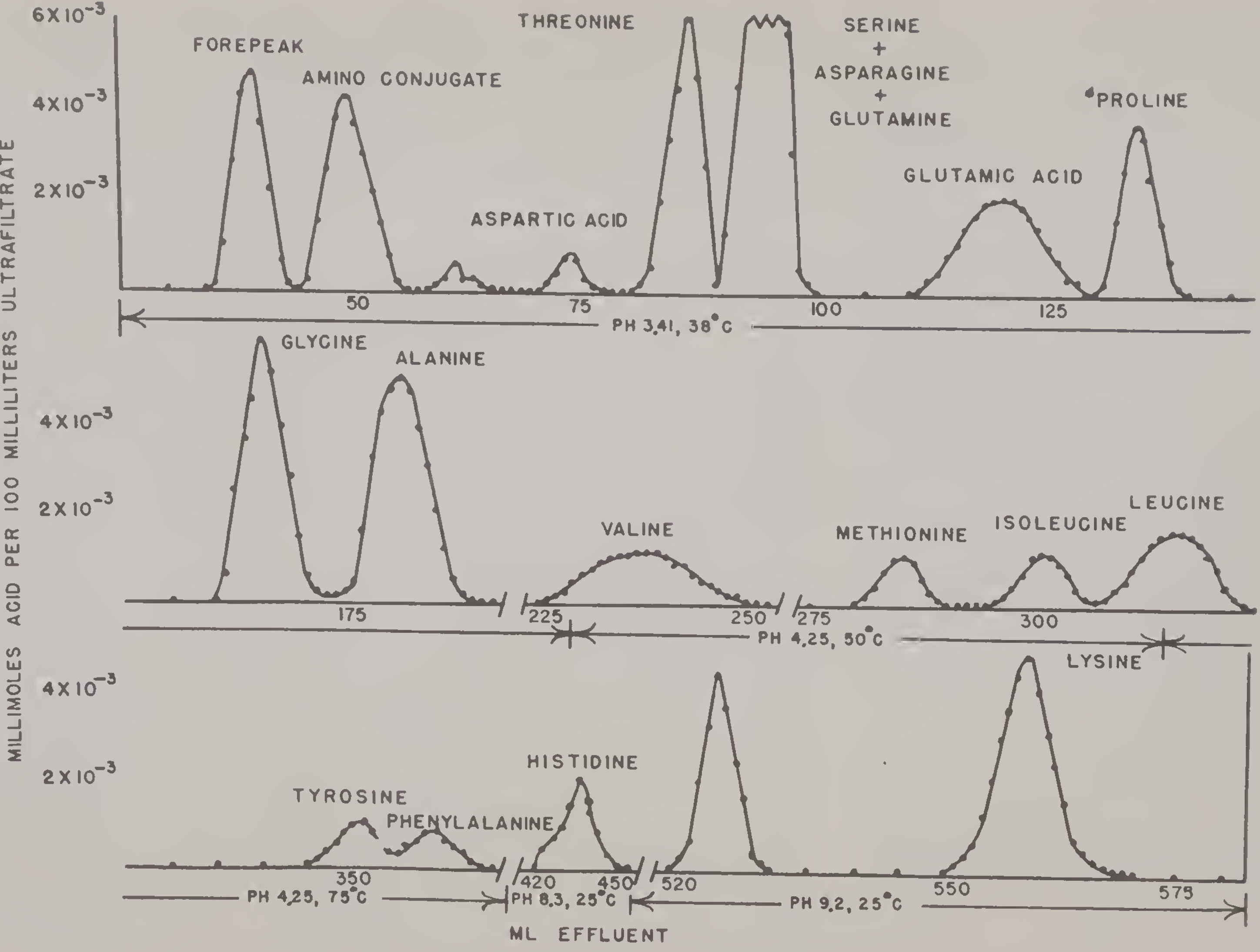

FIGURE 1.—Representative separation of the plasma amino acids obtained by the ion exchange chromatographic technique.

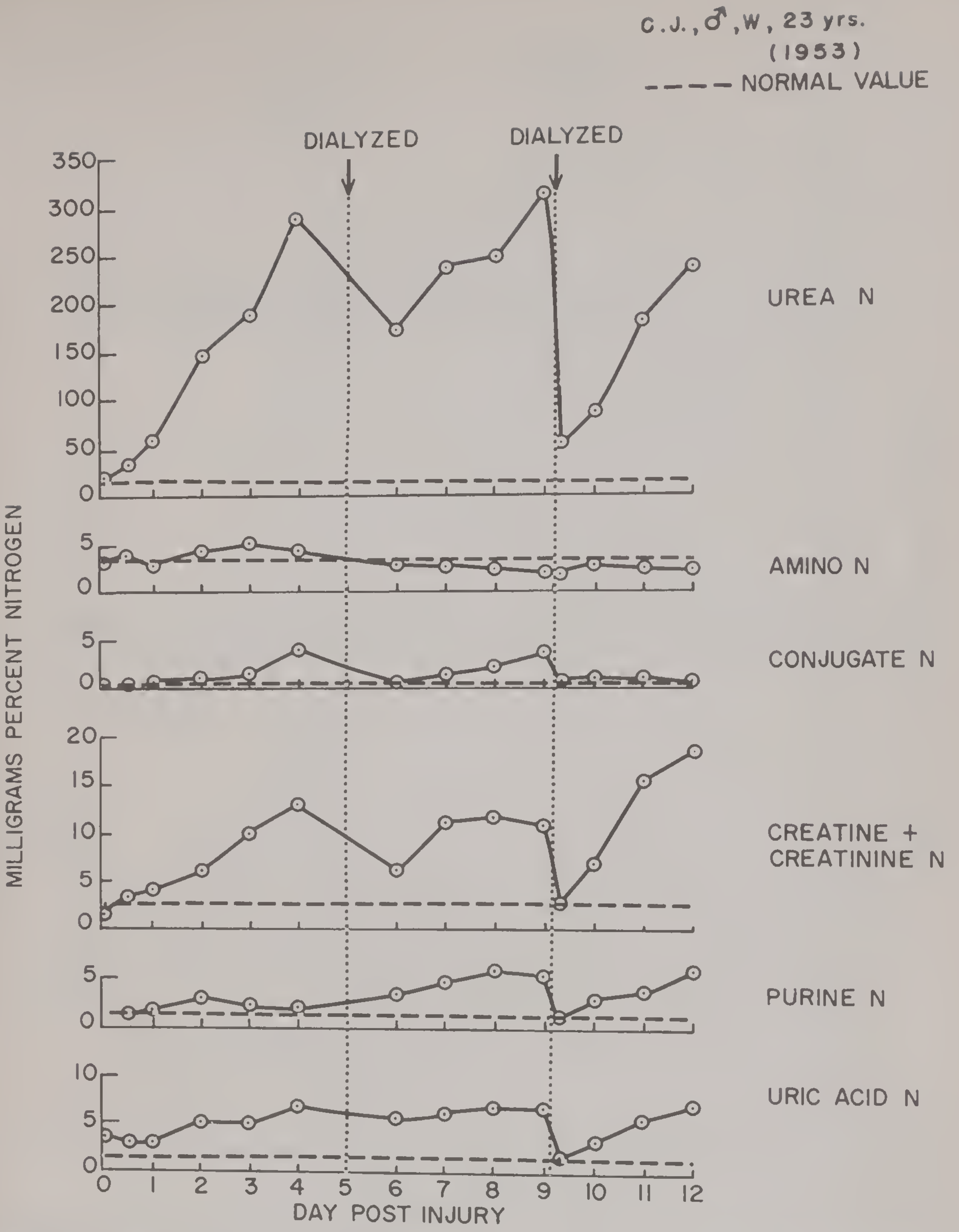

Figure 2.—Plasma nonprotein nitrogen in a patient with severe battle wounds and renal failure.

Among the individual free amino acids in the plasma, proline, threonine, histidine, and glycine stayed near normal concentrations; leucine, isoleucine, lysine, valine, tyrosine, alanine, and taurine rose sharply on the third or fourth day after injury and fell about the tenth day; glutamic acid fell on the third day and gradually returned towards normal (figures 3, 4, 5, 6, 7, and 8). When extracorporeal dialysis was carried out, the levels of the individual amino acids were nearer normal at the end of the procedure (fig. 9) than at the beginning.

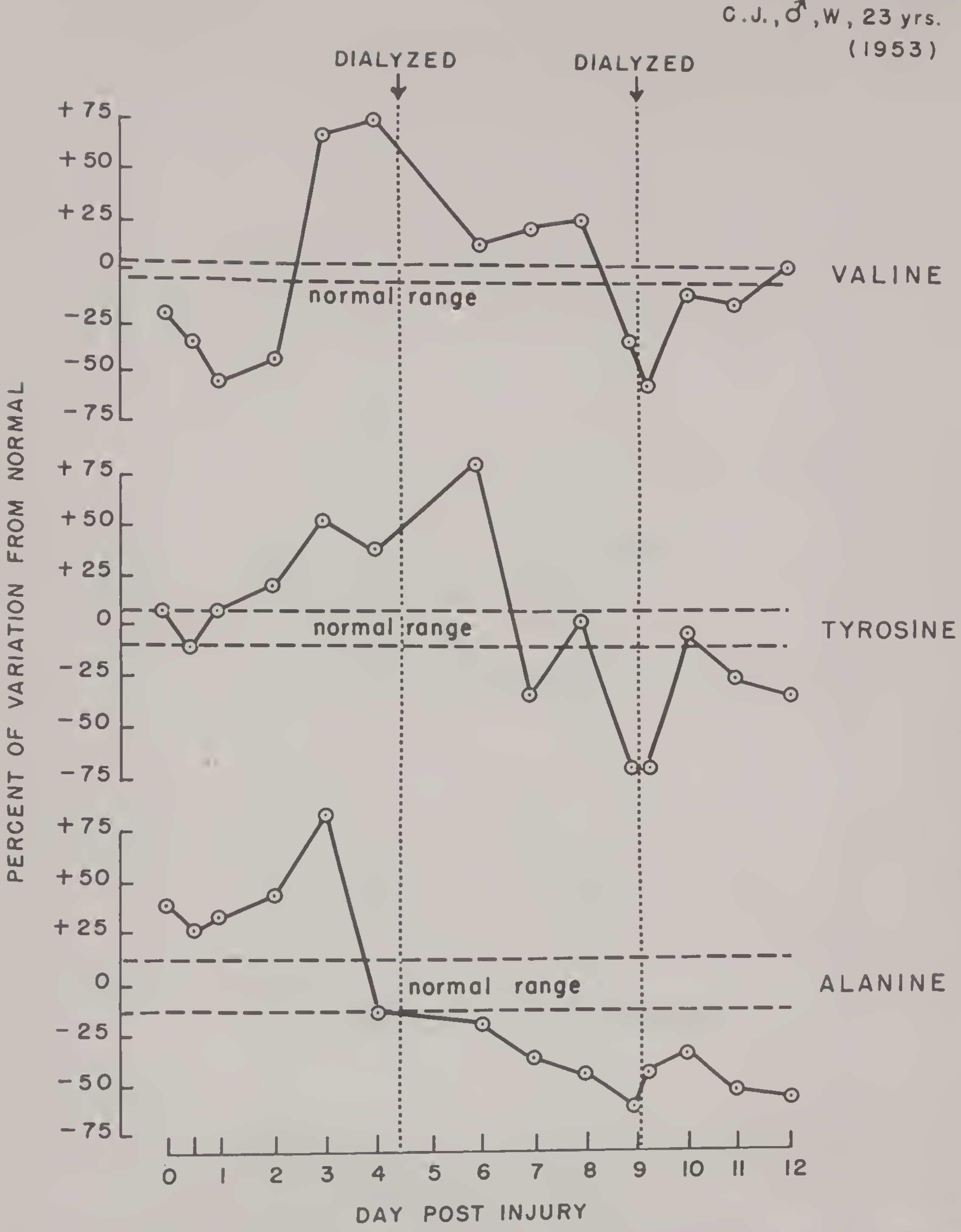

Figure 3.—Plasma amino acids in a patient with severe battle wounds and renal failure (valine, tyrosine, alanine).

Although the total free amino acid concentrations remain near normal, large quantities of an amino acid conjugate characteristically appear in the plasma of patients with injury and renal dysfunction. Early in our work on the plasma ultrafiltrates of human subjects, we noticed that one chromatographically distinct ninhydrin reactive component is unstable to acid hydrolysis. If a plasma ultrafiltrate is heated in a sealed tube with 6N HCl at 110° for 20 hours, the second peak in figure 1 becomes reduced in height. Concomitantly, certain of the other peaks are raised. The acid stable portion of this com-

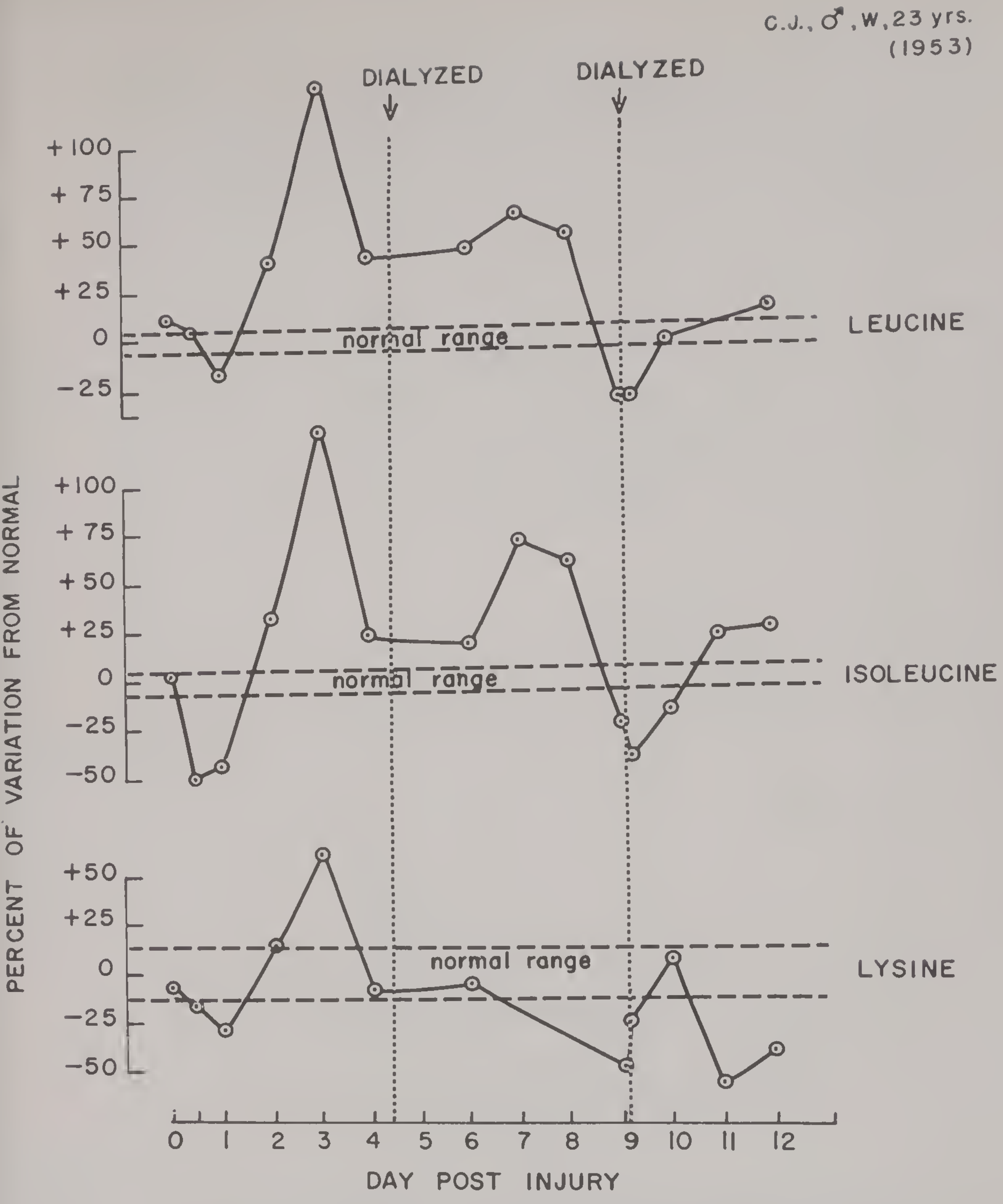

FIGURE 4.—Plasma amino acids in a patient with severe battle wounds and renal failure (leucine, isoleucine, lysine).

ponent is urea; the other is an amino acid conjugate. We isolated, chromatographically, a number of these components, hydrolyzed them as outlined above, evaporated the resultant mixtures of their hydrochloric acid, and rechromatographed them. Some results are shown in figures 10 and 11. The striking central fact is the quantitative and qualitative variability of the composition of this fraction among the patients.

The two normal subjects were young healthy male laboratory technicians. In both, glutamic acid and glycine comprised almost all of the conjugate; a small amount of threonine was also found in one. The plasma of the patients, however, contained the conjugate in greater quantity, and the component amino acids in greater variety.

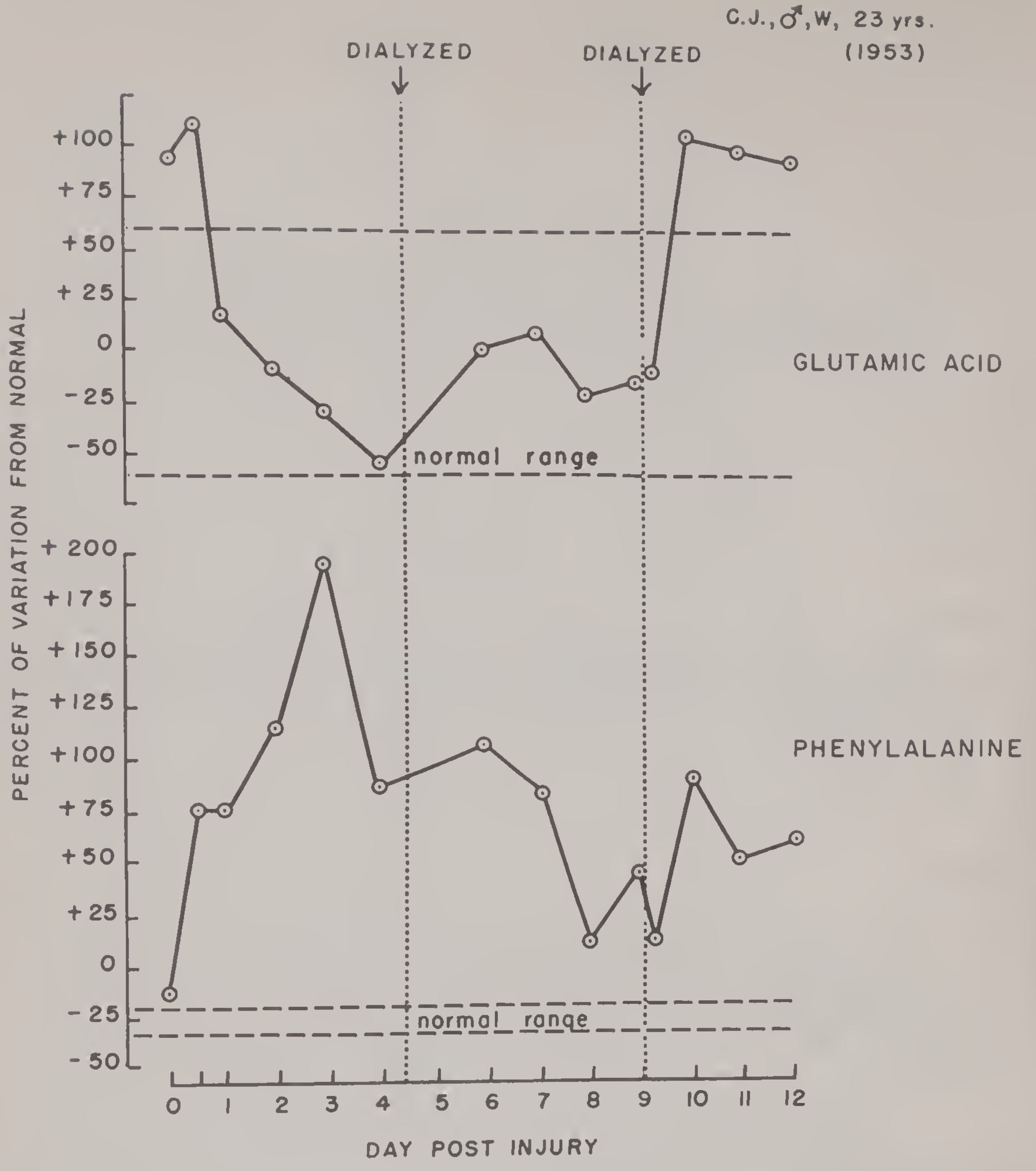

FIGURE 5.—Plasma amino acids in a patient with severe battle wounds and renal failure (glutamic acid, phenylalanine).

In two patients studied (figs. 10 and 11) the plasma conjugate levels rose with the plasma urea levels, though not proportionately. As the level of the plasma conjugate fraction rose, more and more different amino acids were found within the component; the quantitative relations of the amino acids within the conjugate showed an ever shifting pattern, with first one amino acid becoming predominant then another. By the 11th postwound day, in patient C. J. (fig. 10), 12 of the naturally occurring amino acids were present in the conjugate, with leucine and proline in addition to glycine and glutamic acid occupying quantitatively important positions. Patient A. K. (fig. 11) showed the same sort of shifts in amino acid composition, but with different amino acids involved; leucine became even more predominant.

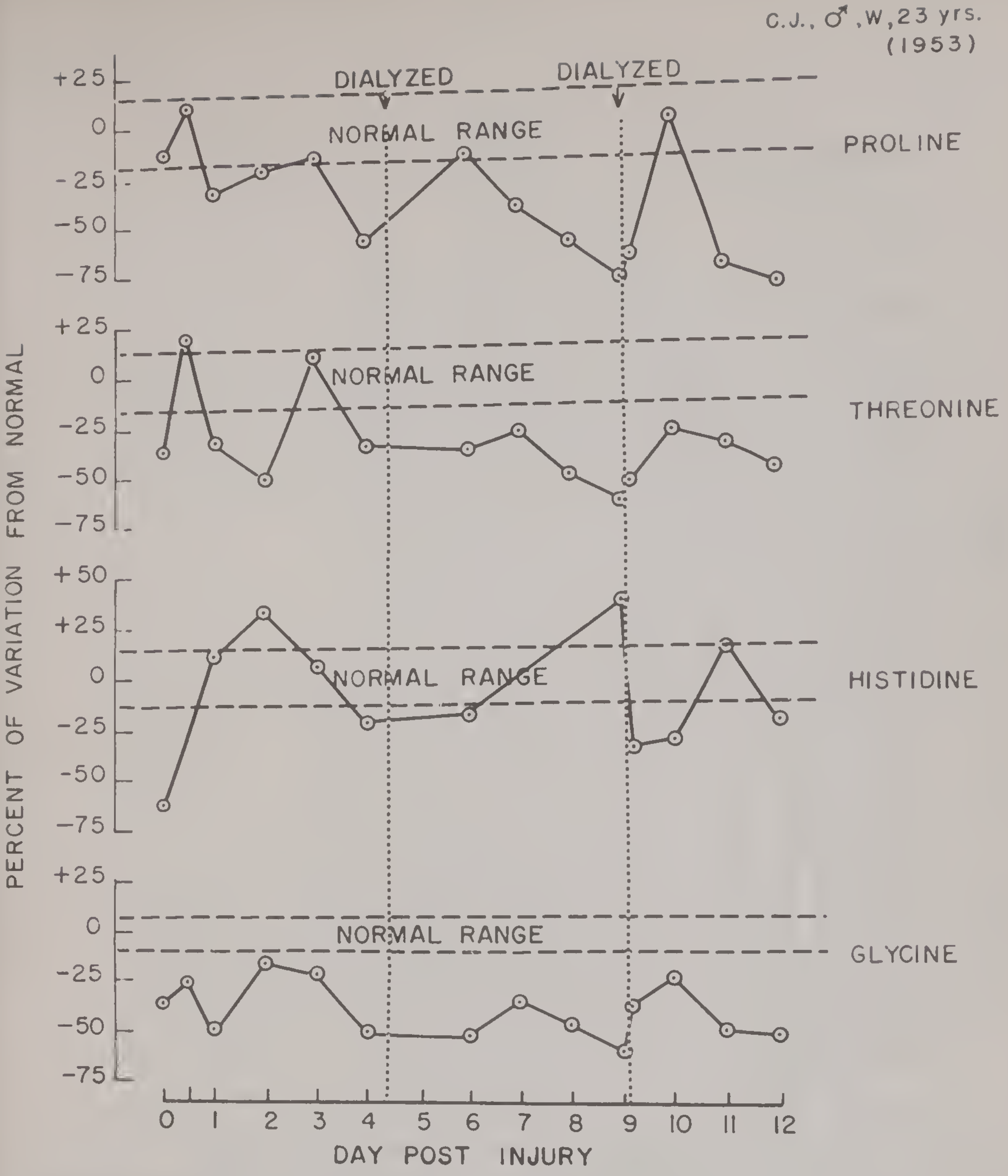

FIGURE 6.—Plasma amino acids in a patient with severe battle wounds and renal failure (proline, threonine, histidine, glycine).

These observations suggested that the amino conjugate fraction is not homogeneous. Accordingly, we chromatographed the intact conjugate on paper by the "circular" technique, using butanol-acetic acid-water (6:1:1) as the solvent. The conjugate of C. J. two days post injury was thereby separated into four ninhydrin reactive components and that of another patient into three. A specific chromatographic analysis was made in one subject (C. J.) for the peptides carnosine and anserine, normal intracellular constituents. Neither was found.

The conjugate, as a whole, acted as a metabolic end product with respect to extracorporeal dialysis (fig. 12). The plasma concentration of the conjugate fraction was reduced after 6-hour dialyses in approximately the same ratios as urea, uric acid, other purines and the crea-

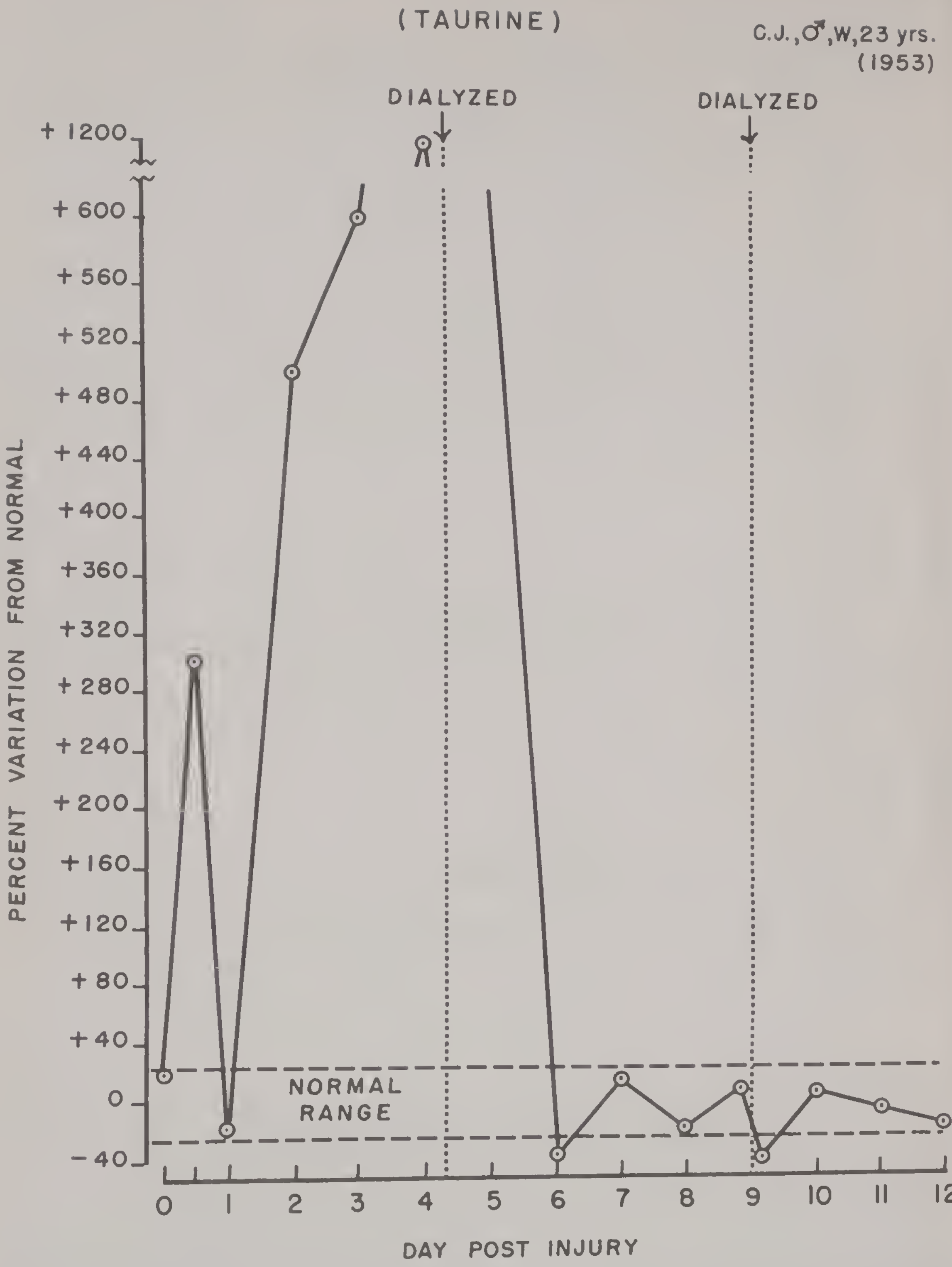

FIGURE 7.—Plasma amino acids in a patient with severe battle wounds and renal failure (taurine).

tine plus creatinine fraction. This is in marked contrast to the behavior of the plasma free amino acids.

Further studies are in progress to determine the nature of this component, its metabolic origin, and its physiologic activity.

From the practical point of view, we know that one of the most important factors which influence the metabolic response to injury and the extent of subsequent nutritional depletion is the food intake. The intake of the seriously injured patient is normally decreased in

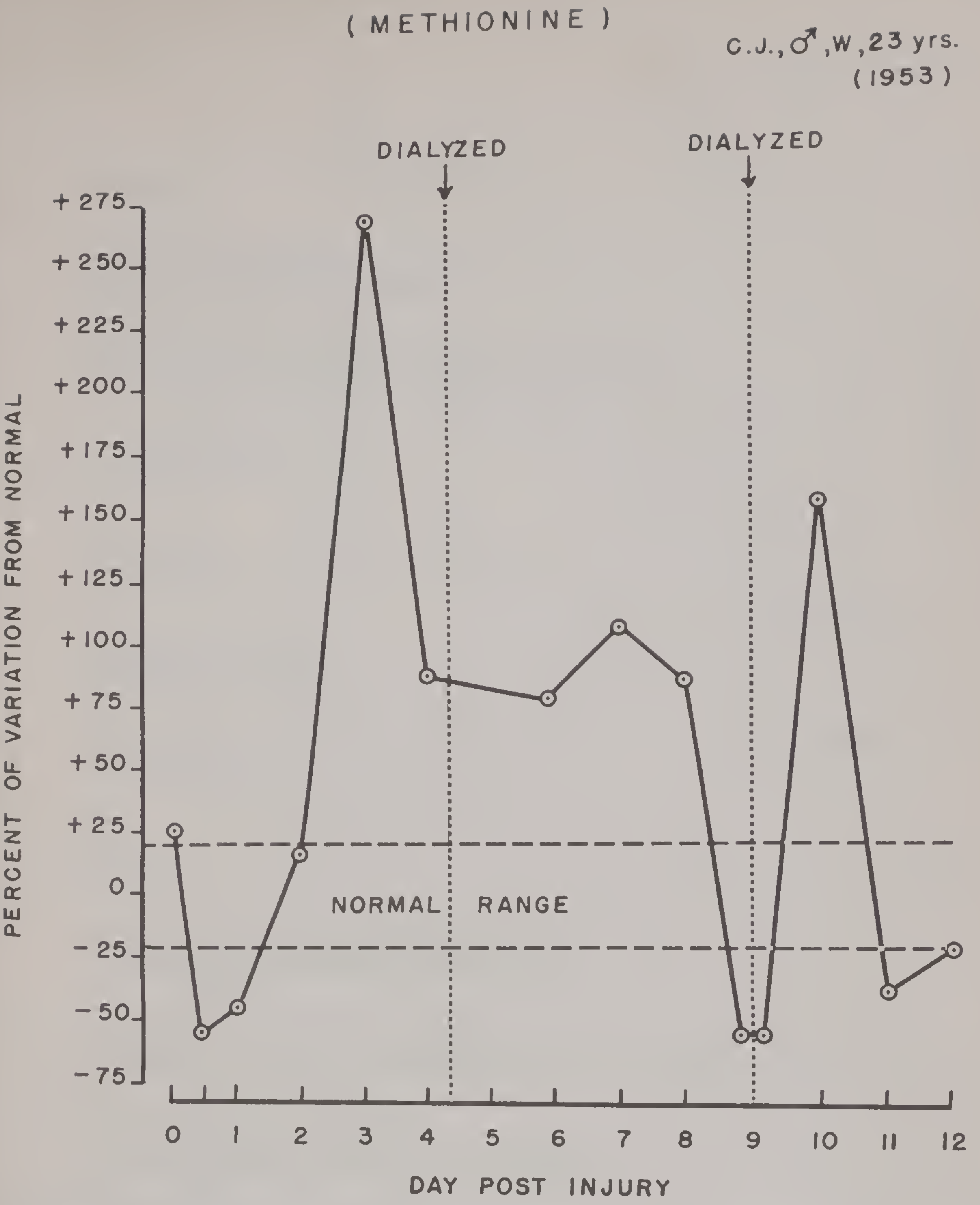

FIGURE 8.—Plasma amino acids in a patient with severe battle wounds and renal failure (methionine).

the early period following trauma and this accentuates the nitrogen loss. If the dietary intake of the seriously injured individual were maintained at an adequate level, beginning shortly after trauma, many of the problems associated with the development of malnutrition would be averted.

Situation at the MASH Level

The following remarks are based on observations made at the 46th MASH during January and February 1953. The majority of patients remained at the MASH for only a few days, and were chiefly on intravenous fluids (blood, dextran, glucose, water, saline) during

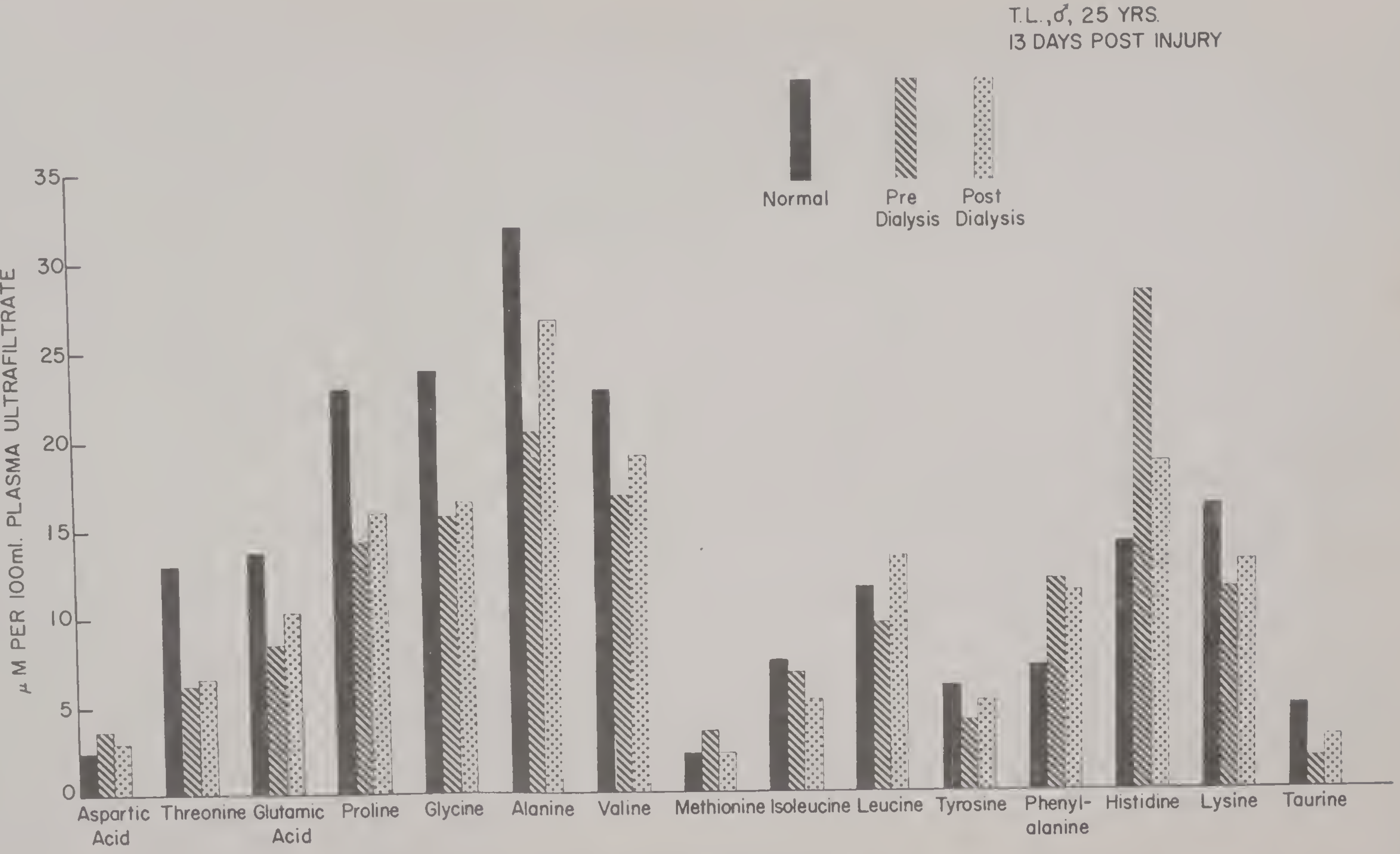

FIGURE 9.—Effect of extracorporeal dialysis on the plasma amino acids of a patient with severe battle wounds and renal dysfunction.

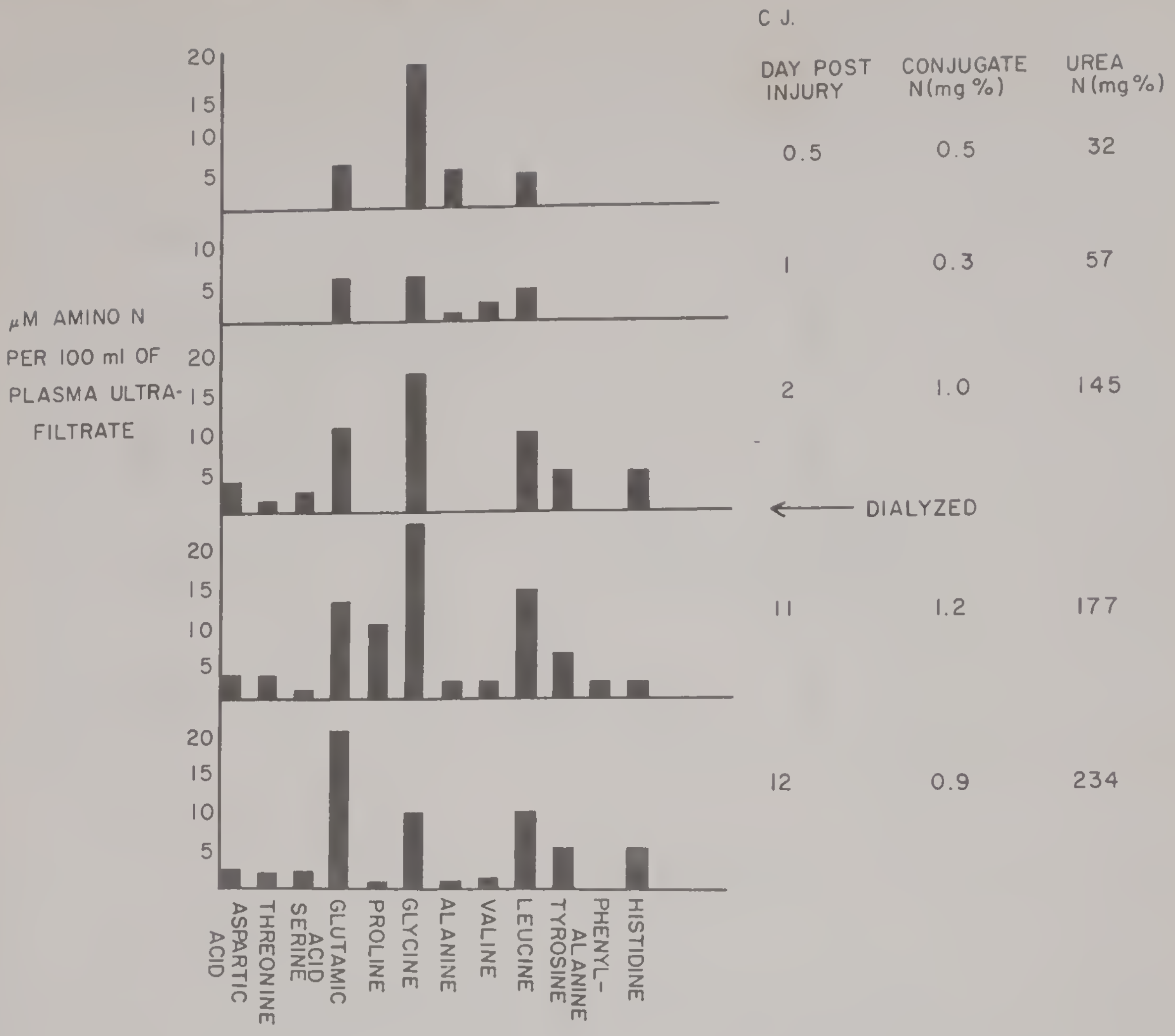

Figure 10.—Composition of plasma amino conjugate following severe battle injuries.

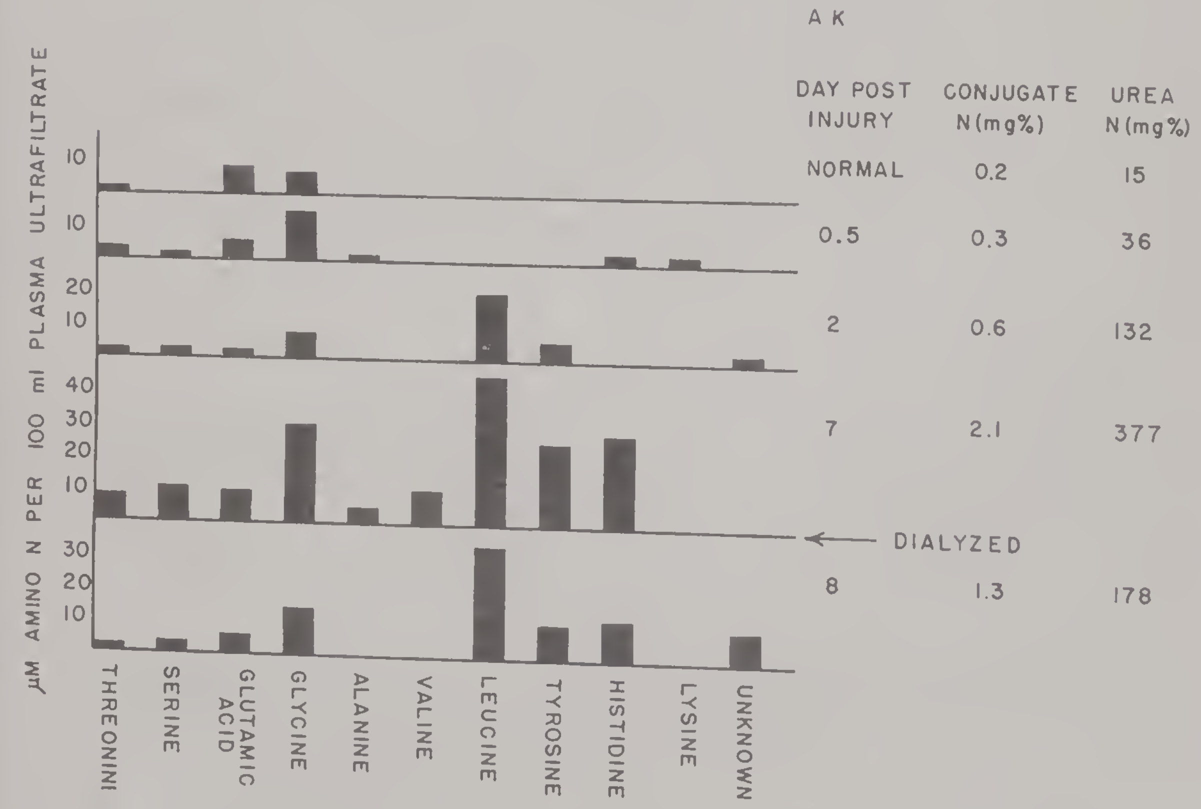

Figure 11.—Composition of plasma amino conjugate following severe battle injuries.

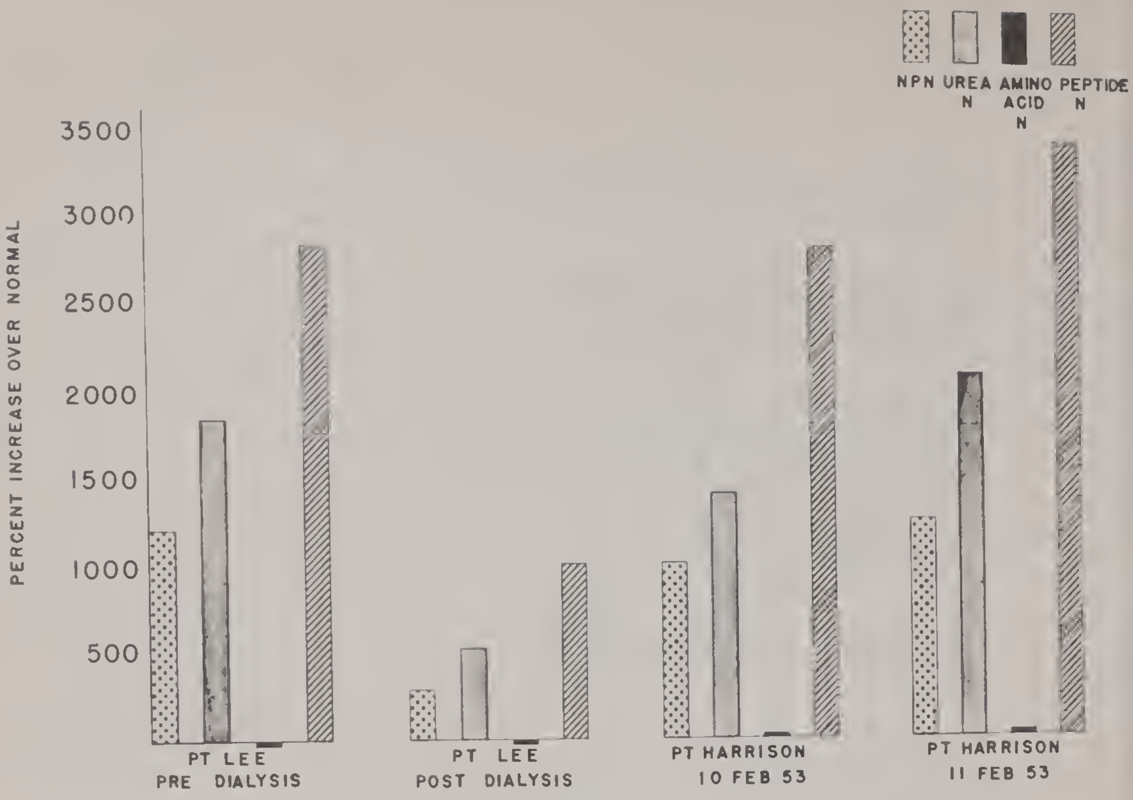

FIGURE 12.—Plasma ultrafilterable nonprotein nitrogen in patients with severe battle injuries and renal dysfunction.

this time. A lesser number of patients remained 7 to 10 days. Adequate nutrient intake was often not ingested by these patients. The patients who ate well were the ones with the less severe injuries; the patients with extensive injuries usually ate poorly.

Vitamins were not given during the preoperative period. In the postoperative period, patients on oral intake were occasionally given one or more multivitamin capsules daily. Those patients receiving intravenous fluids were given 500 mg. of vitamin C and 50 mg. of thiamine once or twice a day. Vitamin K preparations were occasionally given when there appeared to be a specific indication, for example, a decreased plasma prothrombin concentration. This type of vitamin therapy is imbalanced.

Body weights were measured only occasionally, but some patients apparently lost as much as 20 pounds in 10 days. The neurosurgeons were particularly firm in their conviction that serious weight loss occurred very rapidly in their patients.

Experiences at the Renal Center

As already mentioned, serious malnutrition developed rapidly and was an important complication in the patients with severe battle wounds and renal failure. The clinical and autopsy records of the 70 patients admitted to the Renal Center from its opening in the spring of 1952 through the middle of February 1953 were reviewed by one of us (S. L.) with Major Paul Teschan who had been in charge

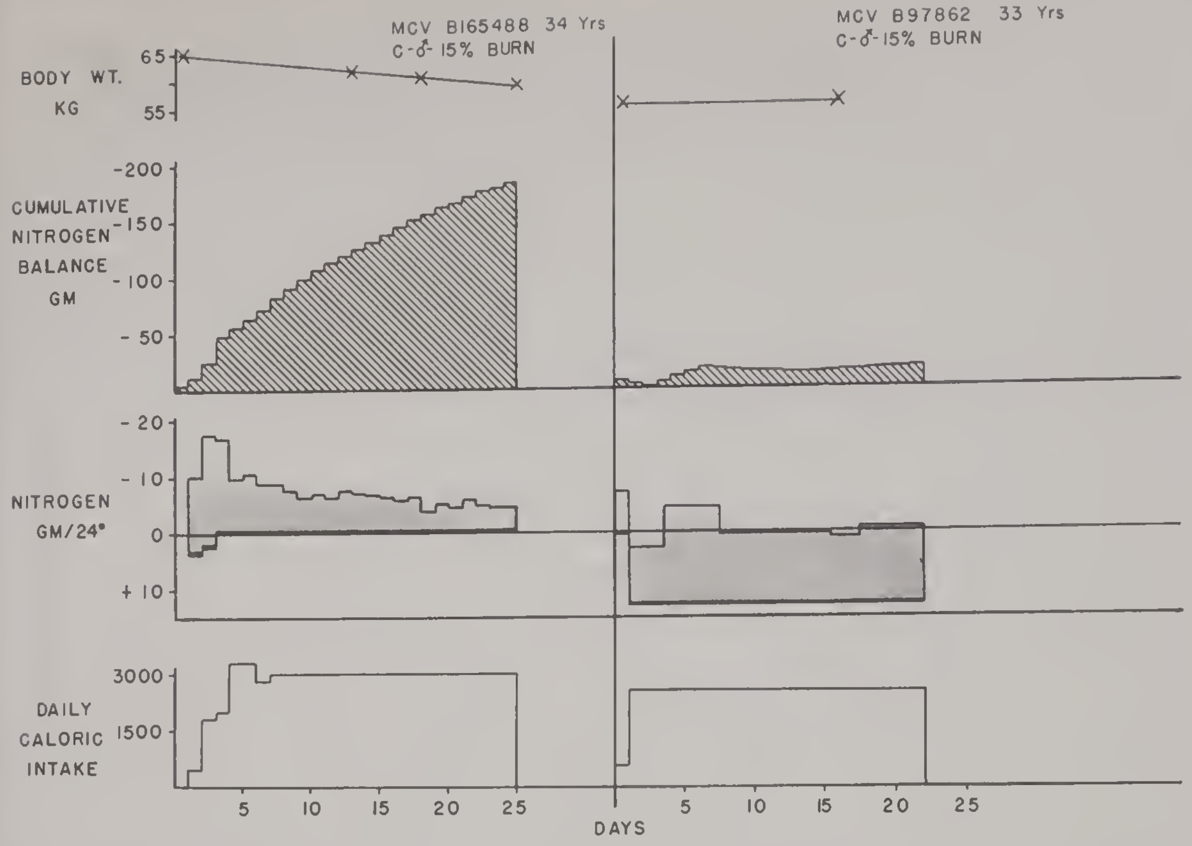

Figure 13.—Nitrogen metabolism following burns. Effect of protein intake.

of the Renal Ward during most of this period. Most of these patients lost weight rapidly. Wound complications and mortality paralleled weight loss. What the weight loss specifically represents in terms of body tissue, water, fat, etc., is not known.

The first weights recorded are those on admission of the patient to the 11th Evacuation Hospital. At this time, many of the patients were presumably waterlogged. Insensible water loss must be an important factor in the weight loss of these patients, since prolonged hyperpnea is a common feature. However, examination of metabolic data available in a few patients reveals a large nitrogen "loss" (i. e., NPN accumulation in the body water, NPN lost by dialysis, and NPN excreted in the urine). In one patient, this amounted to about 45 gm. nitrogen per day, which represents the daily breakdown of about 2.5 pounds of body tissue (excluding fat). It is not possible at present to compare this figure with the nitrogen loss by patients with serious injury without associated renal dysfunction—the data are not available. Urinary nitrogen losses of 45 gm. of nitrogen per day (or greater) are not uncommon among young adult males with serious injuries without associated renal dysfunction, but these latter patients have not been on nutrient intakes as low as the patients studied at the 11th Evacuation Hospital. We know that the level of dietary intakes makes a considerable difference in the amount of nitrogen excretion and not nitrogen loss after injury.

This may be illustrated by figure 13 which depicts data on two patients of similar age with almost identical injuries. The injuries were full thickness burns involving about 12 percent of the body surface. The patients were placed on constant diets supplying to each 45 cal. per kg. body weight but no protein was included in the diet of patient A, whereas patient B ingested one-and-one-half grams of protein per kg. body weight. The net loss of body nitrogen by the two patients is strikingly different. The patient ingesting protein net loss was 16 gm. of nitrogen in the first 25 days after injury, while the net loss of the patient receiving no dietary protein was 175 gm. of nitrogen or ten times as much.

Providing an adequate nutrient intake was a very difficult problem in these patients. Many were unable to take food orally over long periods of time. Parenteral alimentation was limited by the policy of rigid fluid restriction during the period of oliguria (which in some cases has persisted for 2 to 3 weeks). The problem was further complicated by the policy of no protein administration during this same period. (One wonders whether in the face of a very low-caloric intake the nitrogen load is any less in those patients not receiving protein versus those receiving some protein.) Consequently, the nutrient intake of these patients was very low. The average daily caloric intake when the patient had been on only parenteral fluids was 500 to 1,000 calories per day—supplied as glucose. Inadequate data are available as to the utilization of carbohydrates by these patients (cf. the "intolerance" to carbohydrates by individuals with extensive injuries) but the rate of infusion has been generally slow. When fluid intake was restricted, the glucose was usually given as a 25 or 50 percent solution by continuous drip through a polyethylene cannula inserted into the iliac vein via the femoral vein.

The only parenteral vitamin preparations which were available were vitamin C (in 500 mg. ampoules), thiamine chloride (50 m./cc.), and various vitamin K preparations.

It was not possible to determine the oral dietary intake of these patients from the available records, but it would appear that the oral food intake was low until long in the convalescent period. Oral vitamin intake had usually been three of the standard Army multivitamin capsules per day.

It is clear that inadequate food intake is one of the important factors in the pathogenesis of the malnutrition observed following injury. We do not yet know what the optimal intake should be for the severely wounded soldier. One of the difficulties is that little objective data of the physiologic and clinical sequelae directly attributable to the early metabolic reaction to injury are available. I was glad to hear Dr. Allison mention that he was beginning to follow the healing of experimental wounds as a technique for studying certain nutritional problems.

We, of course, are vitally interested in this approach since the injured man must heal his wounds for successful recovery; systematic observations of the healing of wounds, traumatic, operative and experimental, would provide objective evidence in one important area as to the significance of the "catabolic" period. For example, the seriously injured individual acts biochemically like a scorbutic in the first days and well after injury; does he also act like a scorbutic in regard to the healing of his wounds? Further, the intensity of the urinary nitrogen loss following injury may be decreased by the injection of testosterone propionate. Since the anabolic effects of testosterone are different for different tissues, what does the decrease in urinary nitrogen excretion mean in terms of wound healing?

It has been postulated by some that no attempt be made to reverse the "catabolic" reaction because it is a "defense mechanism" to supply metabolites to the injured area. There is no concrete evidence to support this. There is no reason, at the moment, to assume that the injured area is necessarily more proficient than other tissues in "utilizing" the circulating metabolites. We have recently studied the healing of experimental laparotomy wounds in normal and severely burned rats. Observations of the gross appearances, tensile strengths and histologic features of the incisions were made. The healing of laparotomy wounds in the burned rats was significantly different from that in the unburned controls. Epithelization was not affected, but there was a definite delay in the formation of granulation tissue in the incisional wounds of the burned animals with a lag in the appearance and maturation of the fibroblasts and the ground substance. The eventual number and amount of these two elements, however, did not appear to be affected, and abundant granulation formed in the wounds of the burned rats in time.

Summary

In summary, during the recent Korean conflict malnutrition was an important complication in the severely injured soldier with acute renal failure. Since a disturbance in the metabolism of one or more amino acids might be one of the factors in the pathogenesis of the nutritional deterioration, observations of the plasma amino acids were made in some of these patients, using an ion-exchange chromatographic technique. Preliminary data indicate the total nitrogen concentration of the free amino acids in the plasma remained close to normal, in spite of extremely high NPN's. Similarly, the total free amino nitrogen concentration was little affected by extracorporeal dialysis for 6 hours.

Among the individual free amino acids in the plasma, proline, threonine, histidine, and glycine stayed near normal concentrations; leucine, isoleucine, lysine, valine, tyrosine, alanine, and taurine rose sharply on the third or fourth day after injury and fell about the tenth day; glutamic acid fell on the third day and gradually returned towards normal. When extracorporeal dialysis was carried out, the levels of the individual amino acids were nearer anormal at the end of the procedure than at the beginning.

Although the total free amino acid concentrations remained near normal, large quantities of an amino acid conjugate characteristically appear in the plasma of patients with injury and renal dysfunction. The striking central fact is the quantitative and qualitative variability of the composition of this fraction among the patients. The conjugate, as a whole, acted as a metabolic end product with respect to extracorporeal dialysis.

It is clear that inadequate food intake was one of the important factors in the pathogenesis of the malnutrition observed in these patients. We do not yet know what the optimal intake should be for the severely wounded soldier. One of the difficulties is that little objective data of the physiologic and clinical sequelae directly attributable to the early metabolic reaction to injury are available. We are vitally interested in following wound healing as a technique for studying certain nutrition problems. Since the injured man must heal his wounds for successful recovery, systematic observations of the healing of wounds, traumatic, operative, and experimental, would provide objective evidence in one important area as to the significance of the "catabolic" period.

Acknowledgments

The study was made possible through the efforts of Colonel William S. Stone and Colonel Richard P. Mason. We thank Dr. John M. Howard, Major Paul Teschan, Major William H. Meroney, Lieutenant Patrick O'Meara, Lieutenant Robert Abernathy and other members of the Surgical Research Team and the Eighth Army Medical Corps at the 46th Mobile Army Hospital and 11th Evacuation Hospital in Korea who were responsible for the care of these patients and made available to us the patient data and plasma samples. The chromatographic analyses were carried out by us in the Department of Surgical Metabolism, Army Medical Service Graduate School.

Discussion

CHAIRMAN POLLACK:

I would like to ask Dr. Johnson whether in the group of stress subjects they included the oxy-steroids?

JOHNSON:

That is correct. We made sporadic tests of 11 oxy-steroids. It is quite possible they would be a better index. The 17 ketos are the only ones we have routinely on all subjects.

JOHN B. YOUMANS (Vanderbilt University):

Dr. King, who opened the discussion yesterday, looked backward and brought into focus what has been done previously, what the status of affairs was some years ago. He pointed out the deficiencies, the questions we needed to solve, and the line of work which should be pursued in order to make a proper advance. It seems to be my duty to consider what has been said today, to see how far it goes toward meeting the objectives outlined by Dr. King, and to project into the future, the work which must be done to go still further forward in this field.

It is quite obvious that I can't review each paper in detail, particularly on the basis of the specific objectives which each had. It seems to me that what I must do is look at the symposium as a whole, to see how well it has fulfilled its objectives.

You will remember that the symposium is on the subject of "Methods for Evaluation of Nutritional Adequacy and Status." We must therefore consider what that means in more specific terms and keep in mind that it means adequacy of diet, adequacy of diet in terms of individual nutrients, adequacy of diet for test animals, and for man—and also means nutritional status and how to evaluate it for both animals and man, not only under relatively normal conditions, but under conditions of the most severe stress and strain, both physical and mental.

Finally, we must keep in mind that the particular concern of the Armed Forces is with nutritional status and behavior under stress and strain and our ability to determine it or to assess it under those conditions.

There have been many papers in the symposium. So much of the work reported is, of course, not directly concerned with the specific objective of the assessment of nutrition and nutritional status. But, it all points up in that direction. Certainly it is no deprecation of the work with animals or bacteria, or work of any other kind, to say that from that work we go forward to determining these situations in man, and in particular, in the personnel of the Armed Forces.

We started out our program, I thought, with a very significant discussion by Dr. Crampton, who of course, reminded us of the importance of the use of biostatistical techniques and procedures in our work. I think we are arriving finally at the position in which we call in the architect for the building of our experiment before we start the building and are guided by his recommendations in order that the house may be most sound, effective, and perhaps beautiful.

There has been, I think, some deficiency on the part of the statisticians. I am not at all sure they were, in the beginning, equal to providing us with proper plans in certain fields, which were somewhat new to them. I refer particularly to the studies of nutrition of populations, in which there seemed to be a considerable difference of opinion as to what the statistical limits and landmarks were.

It would seem to me that now, not only do we have them in better shape in regard to what we might call standard and better-known biological experiments, but also in regard to these complex and newer methods of estimating the nutrition of populations.

This matter came into focus again in connection with Dr. McGanity's paper which essentially was a plea for critique in the use of a method which is notoriously open to subjective error; error of the individual, and, I think, emphasized again the importance of proper procedural and statistical control.

It seems quite clear we have gone a long way with regard to protein, and are opening up new vistas with regard to amino acids and their part, not only in nutrition, but the part they can play in interpreting for us the function of protein, the symptoms and signs of protein deficiencies and errors in protein nutrition.

I think there is still a place for studies of total protein in the quantitative sense and, to give a single example, the point made by Dr. Cannon of the surprisingly large amounts needed for repletion is indicative of that point.

It appears, however, that we are going to go much further in the field of the enzymes and the influence on nutrition of enzymatic processes in our understanding of many of the physiological problems which have been facing us. Here is one of the few instances in which the threat of a possible deleterious effect of overnutrition appears.

I am not enough of an enzymologist to be able to judge correctly or discuss in detail Dr. Snell's reference to the production of excessive amounts of enzymes, and the possible influence they might have on our physiological processes. But, I have for some time been questioning in my mind, the ultimate result of increasing—I don't like to use the word "better," but I will for the moment—better nutrition. Better nutrition was discussed by Dr. Sebrell this morning in relation to the increasingly rapid rate of growth in children. You can do it in animals, too, of course. We all are familiar with the fact that the size of individuals in our population and the rate of growth of the young, at least in this country, have increased over the decades. The question remains, however, whether that is advantageous, and certainly one can get some evidence which I won't attempt to give in any detail today, that this may have an influence on the factors of longevity and the aging process, an effect on maturation, which may in turn be an indication of and a factor in relation to aging and longevity. That brings us again to Dr. Carlson's remark regarding the use of life span in animal experimentation as a measure of the effect of various types of nutrition.

We seem to have gotten to somewhat of a plateau with regard to our understanding and use of excretion tests, particularly of the water-soluble vitamins, and blood levels of certain vitamins. Perhaps the biggest gap at the moment is our failure, and I think it is regrettable, to have arrived at better standard techniques. Each laboratory can make studies of this kind and arrive at conclusions which are satisfactory to them under their circumstances and which we will generally accept as valid under the particular circumstances. But, when one attempts to apply that on a larger scale and wants a larger volume of evidence to use, he finds that he has difficulty in picking from this man's work and that man's work, tests of sufficient similarity so they apply to the larger problem which he has in mind.

I think most of us would agree now that the levels of excretion, both with and without test loads and the levels of concentration of certain nutrients in the blood under proper conditions may be used with proper critique and discretion as indicators of nutritional status. If we do disagree at times in individual instances, I think that if we will clearly state just what we had in mind, just what we expected to get from it, we will not have the disagreements we were having 10 years ago with regards to the significance of some of these tests.

The discussion of mineral adequacy was very interesting to me because it again may have forced some revision in our thinking with regard to the significance of the size of intake of what seem to be desirable nutrients; the difference between behavior on the basis of a customary lower intake and the basis of a customary high intake of calcium, and in some cases, the difference in the composition of the body which resulted under those two conditions. I refer, of course, to the changes in character of the bony skeleton. I think we might bring into this same picture, the changes which Dr. Levenson spoke about a moment ago, and which Dr. Allison discussed, in regard to wound healing. You will remember that when the wounds were healed under conditions of poor nutrition, they didn't heal as well, but the tensile strength was greater. When they healed faster under conditions of improved nutrition, although they showed better evidence microscopically and histologically of healing, the tensile strength was less. That is a reversal of a trend which I think may be of some significance. It is a small straw in the wind to go along with the changes that occurred in the skeleton on lower levels of intake of calcium.

Let us point out the conditions of the bones—a somewhat lesser density of bones with a high intake and a high turnover than you would get with a lower intake and a slower turnover. You are perfectly free to disagree with me.

The rat, monkey, and chick work, as I have indicated, cannot in any way whatever be slighted. The type of experiment carried on by Dr. Elvehjem is typical of the thing which must be done in order to give us leads with regard to human metabolism. However, I think you will all agree with me when I say that although we do get very important information and leads from these experiments, there is the error of attempting to carry over completely or too rapidly, the results of experiments on one species to another. We are all familiar with that danger, but the mistake is quite commonly made, even yet. There is a tendency to extend knowledge to a too great length to the place where it becomes so tenuous it breaks, and that again is one of the aspects in which critique is highly desirable. It is also an area in which judgment becomes of the greatest importance.

With regard to the surveys of population, we seem to have come again to a situation somewhat similar to that of the excretion of vitamins and blood levels of vitamins and their significance for nutrition. We have been carrying on in the world rather broad scale surveys of the nutrition of populations for about 15 years. If I am not mistaken, it was about 1938, at the meeting of the Nutrition Institute in New Orleans, when reports were made regarding 1 or 2 of the very early population surveys. Since that time, they have been done repeatedly and extended in this country. They have been carried on abroad. We have gone through two wars. We have used it for an estimate of the nutrition of troops, both under conditions of garrison and conditions of combat, and we have arrived at a pretty good understanding of the possibility, the limitations, and the values of this type of procedure.

I feel that we have now reached about the limit in certain respects with regard to these surveys. To give you an example, it is unlikely we will ever be able to overcome the disposition to hide the source of food of an individual—for an individual to hide his source of food in a time of short food supply. As Dr. Sebrell mentioned this morning, we will probably never overcome that psychological, that protective reaction. On the smaller scale of physiological experiments, we will never probably be able to do away with the interference of intake which comes with putting an observer in the home. If any of you ever tried to record what you ate, you will realize the problem you have, the hesitancy you have to eat something because you have to write it down and these factors will also limit and interfere with certain of the procedures and techniques which are used in the survey of populations.

Nevertheless, we have, I think, learned much about the errors. We have learned the values. We have learned the techniques. We have trained personnel and we can use such procedures to advantage as long as we keep their use within the limits of what we know is proper. The same thing can be said of the physical examination and laboratory tests—not the kind reported this morning and afternoon by Dr. Johnson, but the kind reported by Dr. Jolliffe, the more clinical, the quick type. We have learned our limitations. We have learned what we can do and what we can't do. They will continue to be useful tools, improving only as we get additional information from other studies such as those that have been presented here and as more basic information comes along which we can incorporate in our clinical procedures and techniques.

Dr. Johnson's and Dr. Levenson's papers indicate the two-way flow of ideas which make for progress. Dr. Johnson, as I have already indicated, and Dr. Levenson, and people like them, incorporate in their studies the things that are fed up to them from the animal experiments, from the more particularized individual experiments on humans, from the work with bacteria.

On the other hand, experiments like those of Dr. Johnson and Dr. Levenson, feed back to the more basic problems, the questions which are raised and they can answer when the question is asked, when they study these people. So, we have here the two-way exchange which, as I say, makes for progress.

Finally, we have the paper by Dr. Brožek, which points out one of the principal objectives of this symposium and the principal responsibility and objective of the Armed Forces, and that is performance. As he has said perhaps the best test of the football player is to see how he performs on the football field, and I am sure that the best test of performance and ability of a soldier, sailor, or airman is how he performs his particular function. It is rather interesting to note that as warfare becomes more mechanized, at least three of the attributes which he mentioned, that is speed and accuracy and coordination, become more important than some of the grosser indices of performance, and that again is a thing with which the Armed Forces have particular concern.

It seems to me that we are having more opportunity to do this testing under conditions which approach the actual playing field or battlefield. Although I may be wrong, maybe I am getting a little softhearted, but it seems to me that the Armed Forces, those who control these things, are more receptive to our feeling that in the end a good many of these things must be tried, must be determined under conditions as close to the actual game as possible. We are approaching that situation.

In closing, I can't help but comment upon what I think is the highly successful outcome of this symposium. I and many others here have been watching and working with this thing for 15 or more years and have been pretty closely connected with the specific purposes of this symposium. I think that this symposium has marked a very significant advance in all lines of that kind in which we are interested.

VII. Round-Table Discussion on Body Composition

Morton I. Grossman, *Moderator*

Aspects of Nutritional Status Reflected by Body Composition

Morton I. Grossman

Medical Nutrition Laboratory

The apothegm "you are what you eat" might be taken as the theme of this panel discussion on Nutrition and Body Composition. But, like most maxims, this one at once says too much and too little, at least insofar as it applies to our topic. It says too much in that diet is not the sole determinant of body composition. Physical activity and genetic factors have an important effect. It says too little in that some dietary abnormalities are not reflected in a change in major body constituents but produce clinical signs or symptoms or biochemical changes which are better indices for their detection.

Nutritional status is a complex concept, not measurable by any single criterion. The new methods for measuring body composition provide much greater precision in the description of nutritional status, especially the caloric aspect. However, even this one aspect has complex relations to general nutrition. Deficiency of protein or vitamins, as well as deficiency of calories *per se*, may lead to caloric undernutrition. On the other hand, some deficiency diseases, such as scurvy or pellagra, may develop in persons with normal body weight. Persons in good health exhibit a wide range of values for height and weight. Information required for the establishment of "norms" for body composition (e. g., percent body fat) are only now beginning to be accumulated.

A new parameter has been added to nutritional studies in recent years, namely, the measurement of the major components of the body and the application of the methods for their determination to human subjects. For many years height and weight have been the principal criteria of caloric nutrition. It is now possible to get information in living subjects on the absolute and relative amounts of the major components which go to make up this height and weight.

What are these major components? Water, mineral, fat, and protein are the major constituents of animal bodies in chemical terms. As yet, methods are available for the direct determination in living subjects of only one of these, namely, water and its various compartmental subdivisions. However, by the application of certain well-validated hypotheses it is possible to make reasonable estimates of the fat content of the body by a variety of methods which will be elaborated upon by the essayists of the panel. Since fat is by far the most variable constituent of the body and the one most clearly related to nutrition, this aspect of body composition will receive major attention in the presentations which follow.

The applications of body component measurements to nutritional studies are manifold and only a relatively small number of the possibilities have been explored in the few years since these measurements have become feasible. Now it is possible to describe obesity and leanness in quantitative terms, the absolute or relative amount of fat in the body. Likewise we can now establish norms for body fat content in terms of actual measurements of fat to supplement criteria of height and weight. Energy requirements and caloric allowances can be related to the composition of the body. The tools have been provided for an attack on the problem of the optimal body composition for longevity, for resistance to metabolic diseases, for physical fitness and for other criteria of good health.

Perhaps an illustration of the application of these methods will serve best to indicate their usefulness. During a field experiment conducted by the Medical Nutrition Laboratory in the winter of 1953, the data presented in table 1 were collected. Observations on total caloric intake, body weight, and percent body fat (by skinfold measurements) were made on 35 soldiers whose body weight changed by less than 0.5 percent during the week of study. Multiple regression analysis revealed that the caloric intake varied with both the body weight and the percent body fat and that the coefficients for both of these terms were statistically significant. It is interesting to note that the coefficient for percent body fat is negative. This indicates that for a given body weight a man with a high percent body fat had a lower caloric intake than one with a low percent body fat. Speculation on the interpretation of this finding will not be indulged in here because these data are presented only for illustrative purposes.

TABLE 1

RELATION OF CALORIC INTAKE TO BODY WEIGHT AND PERCENT BODY FAT IN 35 SOLDIERS STUDIED FOR A 1-WEEK PERIOD DURING WHICH NO MAN SHOWED MORE THAN 0.5 PERCENT CHANGE IN BODY WEIGHT

$\bar{y}$=mean caloric intake=3646 (2548–3997)
$\bar{x}_1$=mean body weight=70.12 (54.05–80.30)
$\bar{x}_2$=mean percent body fat=4.88 (1.8–12.8)
$\hat{Y}=2316.22+22.80\,X_1-55.19\,X_2$
Multiple regression coefficient, $R=0.496$
Standard error of estimate $sy.12=294$
Significance of regression coefficients
For X_1, $t=2.98$, $P<0.05$
For X_2, $t=2.55$, $P<0.05$

The methods for measuring body composition *in vivo* may be divided, for descriptive purposes, into four categories: (*a*) Anthropometrics including body weight, external body measurements, skinfold thickness, and X-ray measurements, (*b*) body density determination by water or gaseous displacement or by gaseous dilution, (*c*) body fat determination by the use of fat-soluble indicators (e. g., nitrogen, cyclopropane, and fat-soluble dyes), and (*d*) measurement of body water and its subdivisions by dilution techniques. A critical appraisal of the principles and techniques of these procedures will be presented by the members of the panel.

A new and powerful tool is in the hands of nutritionists. It is an adjunct to and not a substitute for established methods. Wisely used it can yield important information for the solution of some of the most fundamental problems in nutrition.

Measurement of Body Compartments in Nutritional Research: Comment on Selected Methods[1]

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Introduction

Composition of the body mass shows striking differences between individuals. Compare the grotesquely obese fatness-champion in a circus, with perhaps more than half of his body constituted by fat; the victim of prolonged starvation, with little or no depot fat, grossly shrunk muscle mass, and excess of extracellular body water manifested as edema and ascites; and a patient with severe *anorexia nervosa*, equally "lean" but "dry."

The evaluation of nutritional status, based on height and weight, is not satisfactory even in normal individuals (*9*), when considered from the point of body composition; it may be grossly misleading in patients with a variety of diseases affecting in a differential way the principal components of the body mass.

The elaboration of procedures for a quantitative evaluation, *in vivo*, of the human body constitutes one of the significant developments in the field of human nutrition during the past decade. Since the publication of Behnke's pioneering paper (*2*) a good deal of effort has been devoted to the development and validation of methods for the study of body compartments in the living man.

The task of providing a background for the considerations of the panel on body composition could be approached in a variety of ways. Using the *historical* approach, one could consider the development of the concept of body composition, as a facet of nutritional status and of particular methods used for the measurement of body compartments. One could undertake, on the other hand, a *systematic* classification of the methods that have been proposed by different investigators, and present, in tabular form, the definitions of the basic concepts and the numerical values of the "constants" used in the estimation of equa-

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tions. Also, an attempt at a *critical* analysis of the underlying ideas and mathematical procedures would not be without merit.

In view of the limited amounts of available space this presentation will be focused on a positive yet critical exposition of the logic and mathematics of the methods developed or modified at the Laboratory of Physiological Hygiene, University of Minnesota, with a minimum of reference to the other approaches. Particular attention will be paid to the differentiation between (*a*) definitions, (*b*) assumptions, quantitative in nature, and (*c*) empirically derived values. A comprehensive analysis of the methods for the study of body composition (with special reference to the estimation of body fat in adult man), of the sources of variation in body composition (age, sex, physical activity, and body build), and of some applications (such as analysis of weight changes and definition of optimal reference mass for computations of basal metabolism) have been presented elsewhere (*14*). The positive role in the evaluation of nutriture played by body measurements as well as the limitations of nutritional anthropometry have been discussed recently (*7*) and will not be reviewed here.

In the Laboratory we have strived to develop a theoretical framework for the study of body composition, true with reference to known facts yet internally consistent and allowing a "translation" of the different systems of analysis—hydrometric, densitometric, anthropometric—into one another. The basic concepts are "histochemical," i. e., tissue masses defined in chemical terms of reference. Thus "fat" refers to ether extract, not to the anatomically separated adipose tissue. Even when skinfolds are used they are considered as *indicators* of the fat content. Similarly, the bony dimensions are to be interpreted ideally in terms of bone mineral rather than the skeletal mass. This is preferable but not absolutely necessary, as the description of body composition in terms of anatomical compartments has its justification and usefulness in nutritional research, especially in fieldwork.

There are several reasons for preferring a terminology of macroscopic histology, of chemically defined tissue masses. First of all, the results of the analysis will be more useful in metabolic research. Secondly, an important point is the validation of the procedures for which the direct chemical analysis of cadavers is the final court of appeal and provides the basic data. *In vivo*, the validation of skinfold measurements as indicators of total body fat must be done in terms of correlations with the hydrometrically or densitometrically determined values.

The latter two methods have been developed in terms of tissue masses which are identical (fat, bone) or "translatable" (see table 1). Thus "cells," in the hydrometric approach, represent 30 percent cell solids (19 percent of total body weight) plus 70 percent intracellular water (64−19=45 percent of body weight). The intracellular and extracellular water add up to total body water (45+16=61 percent of body mass). Mathematically, this consistency has been achieved by slight adjustments in some of the coefficients and constants, in the estimation equations, always remaining within the limits of empirically established facts.

TABLE 1

Body Composition of the "Reference Man" (Minnesota System), Based on the Densitometric and Hydrometric Analysis

The mass of the components is expressed as percentage of body weight.

Densitometric approach		Hydrometric approach	
	Percent		*Percent*
Fat	14	Fat	14
Bone mineral	6	Bone mineral	6
Cell solids	19	Cells	64
Water	61	Extracellular fluid	16

In order to round out the picture, before proceeding to a more detailed discussion of the selected approaches, it may be useful to review briefly the procedures which will not be considered in this presentation in detail. These include the following methods in the field of (I) physical anthropology, (II) biophysics, and (III) biochemistry and physiology:

(I) 1. Anthropometric estimation of anatomically defined tissue masses (*19*).
  2. Interpretation of the deviations of standard body weight in terms of "fatness" (*10*).
  3. Measurement of tissue "breadths" from roentgenograms (*26*)

(II) 1. Body volume (and body density) from air pressure changes (*28*).
  2. Body volume from inert gas dilution (*27*), body fatness estimated from combined data on density and body water.
  3. X-ray analysis of bone mineralization (*18*).

(III) 1. Fat-soluble indicators, nitrogen (*1*).
  2. Fat-soluble indicators, cyclopropane (*15*).
  3. Alternative methods for the estimation of body fat from total body water (*17*).
  4. Lean body mass estimated from creatinine excretion and basal oxygen consumption (*21*).

Densitometric Analysis

In a system consisting of two components of known densities (d_1, d_2) the empirically determined value of the density of the system

(D) allows the calculation of the proportional masses of the two components (see table 2).

TABLE 2

THE PRINCIPLE OF DENSITOMETRIC ANALYSIS OF A TWO-COMPONENT SYSTEM

Total mass, $M=M_1+M_2$
Total volume, $V=V_1+V_2$
Density, $D=M/V$. With $M=1$, $M_2=1-M_1$

$$D=\frac{1}{\frac{M_1}{d_1}+\frac{(1-M_1)}{d_2}}$$

Solving for M_1

$$(1)\quad M_1=\frac{1}{D}\frac{d_1\ d_2}{d_2-d_1}-\frac{d_1}{d_2-d_1}$$

Behnke and his coworkers (*25*) have defined the first body component as body fat (with $d_F=0.918$), the second component as lean body mass ($d_L=1.1$). Substituting these two values in equation (1) (table 2), we obtain the well-known equation

$$(1a)\ F=\frac{1}{D}5.548-5.044,$$

except that Behnke has used specific gravity rather than density. The confusion of these terms was unfortunate. One refers to the ratio of mass and volume of the body ($D=M/V$), the other relates the density of the body to the density of water ($sp.\ gr.=D/D_{H_2O}$).

When the density of the water in the tank is used as the reference point, specific gravity can be obtained as the ratio of the mass of the body in air (M) and the mass of the water displaced by the submersed body ($sp.\ gr.=M/W$). The mass of a given volume of water ($W=V\times D_{H_2O}$) is a function of density which, in turn, depends on the temperature of the water. The temperature, and consequently the density, of water has varied in different laboratories in which gravimetric determinations have been made (30° to 31° C. in Behnke's work and around 36° C. at Minnesota) and the values of specific gravity cannot be directly compared. It is preferable to carry all the calculations and report the data in terms of density.

The assumed composition of the lean body mass has undergone radical revision with the passage of the years, the "essential lipids" being reduced from 10 percent (*2*) to 2 percent (*4*), without a change in the assigned density.

The need for a revision of Behnke's system became apparent as a result of three studies which, at the same time, made possible the development of an alternative method of densitometric analysis. The first study (*6*) provided data on the body density of the reference standard, defined as a 25-year-old man, with a body weight standard for his height and age. In the second study (*11*) the density of body fat at different temperatures was determined. This provided a needed tool for the third study which analyzed the composition of tissues gained by positive caloric balance over six months without change in the amount of physical activity (Laboratory of Physiological Hygiene, to be published).

The value used in the past for the density of body fat has varied (0.918 in Rathbun and Pace (*25*), 0.93 in Behnke *et al.* (*4*)). Fidanza *et al.* (*11*) obtained the average value of 0.9000, with the density of samples from five individuals varying within narrow limits (subcutaneous fat from 0.8992 to 0.9009, the internal fat from 0.8998 to 0.9004). These values refer to ether extract at 37° C. For the average temperature of 36° C., at which the fat may actually exist in the body, the density of fat is somewhat higher ($d_F=0.9007$). Using this value and the two other values noted above in Behnke's formula (table 3) we obtain for the "normal young man" ($d=1.063$ or specific gravity of 1.0695 at 35° to 36° C., cf. Brožek (*6*)) the range from 15.8 to 20.1 percent of body weight accounted for by fat. Thus the importance of using a correct value of d_F is quite clear.

TABLE 3

EFFECT OF THE ASSUMED DENSITY OF BODY FAT ON THE ESTIMATION OF TOTAL BODY FAT, AS PERCENTAGE OF BODY WEIGHT, IN BEHNKE'S SYSTEM

$$F=\frac{1}{D}\,\frac{d_F\quad d_L}{d_L-d_F}-\frac{d_F}{d_L-d_F}$$

With $D=1.0629$, $d_L=1.10$, and
$d_F=0.9007$, $F=15.8$ percent
$d_F=0.918$, $F=17.6$ percent
$d_F=0.93$, $F=20.1$ percent

In a study on experimentally induced obesity, carried out by the staff of the Laboratory of Physiological Hygiene, it became quite clear that when the men gained weight—under the given experimental conditions—the gain (G) was not fat, definable as ether extract. The mean density of the gain was 0.948 gm./cc. (table 4). This is somewhat higher but of the same general magnitude as the value of 0.937 reported by Behnke, Feen, and Welham *et al.* (*3*) for a tissue lost by a man whose weight decreased from 91.6 kg. in 7 months. The density of weight gains observed in 6 men over a period of 2 years ranged from 0.903 to 0.941 (*4*). In considering the differences between groups of thin and fat individuals, Behnke, Feen, and Welham (*3*) arrived at 0.94 as the density of the tissue which accounts for the difference in the corporeal density.

TABLE 4

Average Density of the Tissue Gained in Six Months of Positive Caloric Balance

Values represent averages for 10 men

(Laboratory of Physiological Hygiene, unpublished data)

	Initial values	Final values	Difference
Body weight, kg	69. 834	81. 275	11. 441
Body volume, liters	66. 168	78. 239	12. 071
Density	1. 0554	1. 0388	. 9478

All these data indicate that the tissues which are gained or lost contain other components than the fat of the density of 0.9007. For purposes of analyzing the composition of the body it is preferable to break down this adipose tissue into its histochemical components. In the Minnesota obesity study it was shown that extracellular fluid accounted for about 14 percent of the total gained mass, leaving a dry gain with a density of 0.9395, consisting of fat and cells which represent about 72 and 28 percent of the dry gain or about 62 and 24 percent of the total gain.

Assuming that interindividual differences in body composition are due to just such tissues, we can define a reference man of standard density and mass (S), and determine in a given individual the mass of his body accounted for by the G tissues or by the fat contained in the G tissues ($\Delta F=0.62G$) (see table 5). The reference man was defined in the Minnesota version of the densitometric method as the 25-year-old man, of standard weight for height and age and the empirically determined density of 1.0629 gm./cc. The values of G (or ΔF) indicate the way in which the individuals differ from the standard and the values can be either positive or negative, defining the individual's relative position along the leanness-fatness continuum.

Table 5

Densitometry, Minnesota System, Estimation of G Tissues and of the Fat Content (ΔF) of G

$$M=S+G;\ \text{with } M=1,\ S=1-G$$

$$\text{For } ds=1.0629,\ d_G=0.948$$

$$\text{(2)}\quad G=\frac{1}{D}\,\frac{d_G\quad d_s}{d_s-d_G}-\frac{d_G}{d_s-d_G}=\frac{1}{D}8.753-8.235$$

$$\text{With } \Delta F=0.62\ G$$

$$\text{(3)}\quad \Delta F=\frac{1}{D}5.427-5.106$$

Most frequently it is the *relative* fatness of an individual, his deviation from the standard, which is of primary interest. The *total* amount of body fat (F) can be estimated having made an assumption, consistent with the known facts, that in the standard reference body (with $d_S=1.0629$) fat represents 14 percent of the weight. In a given individual the total fat is obtained as the sum of the fat contained in the S and G fractions of the body, F_S and ΔF. ΔF represents the amount of fat by which the given person differs from the standard (in which $G=0$ and, consequentily, $\Delta F=0$). The formula for predicting total fat from body density is developed in table 6.

TABLE 6

DENSITOMETRY, MINNESOTA SYSTEM, ESTIMATION OF TOTAL FAT

$$F=Fs+\Delta F$$
$$\text{with } Fs=0.14\ S=0.14\ (1-G)$$
$$\Delta F=0.62\ G$$

$$F=0.14\ (1-G)+0.62\ G=.14+.48\ G$$

$$F=.14+0.48\left(\frac{1}{D}\,8.753-8.235\right)$$

$$(4)\quad F=\frac{1}{D}\ 4.201-3.813$$

There are several reasons why the use of the standard body mass is preferable to the fat-free body mass or Behnke's lean body mass as a reference standard. Perhaps the most important reason is that our reference man represents the central point in the leanness-fatness continuum. Consequently, errors in the estimated fat content of the body, arising from the uncertainties about the composition of the G mass, will be smaller when we start adding or subtracting G from the center than when we are adding it up to the fat-free or lean zero point ($F=F_S\pm\Delta F$ versus $F=F_L+\Delta F$).

In examining the precision with which body fat can be estimated densitometrically, we must consider the variability for the constants of the two components in the system, S and G. This variability is only imprecisely known at the present time, especially as far as S is concerned.

One of the factors which potentially disturbs the validity of the densitometric analysis is the hydration of the body. The equations for the calculation of body fat (whether this refers to ΔF or F) are applicable, without correction, when the body hydration is normal. In the standard reference man the extracellular fluid, measured as equivalent of the inulin space ($E=0.7\ T$, where T is the fluid in the thiocyanate space), accounts for about 16 percent of the body weight (see table 1). An increased amount of extracellular fluid, with its low density, would simulate higher fat content. When measurements of the extracellular fluid are made in conjunction with determinations of body density, the deviations in hydration from the value of the standard reference man may be taken into account in the calculation of the excess fat (ΔF) and the total fat (see table 7).

TABLE 7

DENSITOMETRY, MINNESOTA SYSTEM, CORRECTION FOR EXCESS HYDRATION

With E=weight of the extracellular fluid,
as fraction of the total body weight,

$$\Delta F=\frac{1}{D}5.359-0.366E-4.982 \tag{3a}$$

$$F=\frac{1}{D}4.149-0.283-3.717 \tag{4a}$$

Because of the lack of clarity in the use of the terms "density" and "specific gravity," the definitions and the calculational formulae are given in table 8. If specific gravity is used, the temperature of the water in the tank should be specified so that the values obtained by different investigators at different water temperatures could be compared. The reduction to density, as the basic unit, is recommended. In addition to the problem of comparability, there are two other compelling reasons for thinking and reporting the data in terms of density. Firstly, the theoretical deviations of the estimation equations are based on considerations of the ratios of masses and volumes. Secondly, when body volume is obtained by air displacement rather than by water displacement, it would be a strange and devious procedure to convert the densities into specific gravity values.

TABLE 8

DENSITY AND SPECIFIC GRAVITY

$$D=\frac{M}{V} \quad Sp.\ gr.=\frac{D}{D_{H_2O}}$$

The mass of water displaced by submersed body

$$W=M_{\text{in Air}}-M_{\text{in }H_2O}$$

The mass of the body under water

$$M_{\text{in }H_2O}=M'_{\text{in }H_2O}+V_R\times D_{H_2O}, \text{ where}$$

M'=the apparent mass of the submersed body
V_R=the volume of air in the lungs and respiratory passages at the time when M' was determined

The volume of displaced water (and of the body itself)

$$V=\frac{W}{D_{H_2O}}$$

Substituting the proper values in the equation for density

$$D=\frac{M_{\text{in Air}}\times D_{H_2O}}{M_{\text{in Air}}-M'_{\text{in }H_2O}-V_R\times D_{H_2O}}$$

Using the density of the water in the tank as the reference point

$$Sp.\ gr.=\frac{M_{\text{in Air}}}{M_{\text{in Air}}-M'_{\text{in }H_2O}-V_R\times D_{H_2O}}$$

TABLE 9

EFFECT OF IMPRECISION IN THE VALUE OF SPECIFIC GRAVITY ON THE ESTIMATED AMOUNT OF FAT IN THE BODY

Using in the formula (*25*)

$$F = \frac{5.548}{sp.\ gr.} - 5.044$$

sp. gr. (at 36° C.) $= 1.070$, $F = 14.1$ percent
sp. gr. (at 25° C.) $= 1.066$, $F = 16.1$ percent
density $= 1.063$, $F = 17.5$ percent

The magnitude of the differences in body fat, estimated from the formula of Rathbun and Pace (*25*) when different values of specific gravity are used, is indicated in table 9. Obviously, the effect cannot be neglected.

Hydrometric Analysis

In the analysis of body weight on the basis of the determination of body water the development of the formulae for the calculation of body fat (*14*) has proceeded, in principle, along the path followed by earlier investigators. The merits or, at any rate, the goals of the Minnesota revision revolve around three principal points:

- (*a*) Clarity of the logic involved in developing the calculational formulae; while this does not guarantee the factual accuracy of the system, it should make it more readily amenable to amendment by the use of more valid coefficients and constants.
- (*b*) Examination of the magnitude of the errors of estimate resulting from the interindividual variability of the biological constants utilized in the derivation of the estimation equations.
- (*c*) Establishment of a reference standard consistent with the densitometric model.

Pace and Rathbun (*24*) considered the body mass (M) as a two-component system $M = F + L$ where F = fat and L = lean body mass, of relatively constant composition. Assuming a value (k) for the water content of the lean body mass ($A = kL$), we can estimate the latter when the total amount of body water (A) had been determined empirically ($L = A/k$). Fat is obtained by difference, $F = M - L$. Expressing all the values as fractions of body weight and using the derived value for L we obtain $F = 1 - A/k$.

Pace and Rathbun (*24*) applied to man the average value of $k = 0.732$, derived from literature and their own data as the average fraction of the *fat-free* body mass represented by water in rats, guinea pigs, rabbits, cats, dogs, and monkeys. Osserman *et al.* (*23*) assumed the value of fat as was estimated from specific gravity in a sample of 81 male subjects. This procedure can be justified, provided the rationale is stated clearly. McCance and Widdowson (*17*) used

$ak = 0.71$. Direct analysis of the two most normal bodies analyzed *in toto* so far indicates 68.4 percent of water for a 46-year-old man and 73.2 percent for a 42-year-old woman (see Forbes *et al.* (*12*); Widdowson *et al.* (*29*)); both values refer to the percentage of fat-free weight. Whatever be the average value we wish to use—the equation of the type $F = 1 - kA$ can be applied only to individuals with a normal composition of the fat-free weight. This point has been emphasized by McCance and Widdowson (*16*) who developed a more comprehensive breakdown of body weight in which body fat is obtained as body weight minus the weight of the extracellular fluid, the cell mass, and the minerals. The frequent occurrence of edema in nutritional deficiencies, notably in starvation (*13*) demands that the state of dehydration be taken into account and the estimate of fatness be made more precise by the determination of extracellular fluid in addition to total body water.

In the Minnesota version of the hydrometric analysis of body composition (*14*) the starting point is the separation of the total body mass into fat, water and the two types of nonfat solids, the dry cell residue and the bone minerals. The definition of symbols and relationships is given in table 10. Our aim is the determination of F defined as (5) $F = M - A - S - B$. We measure M, the body weight, and A, the total body water, and estimate the two nonfat solid compartments, S, the cell solids, and B, the bone mineral. The quantitative assumptions and the derivations of the computational formulae are summarized in table 11.

TABLE 10

Hydrometric Analysis: Definitions

Definitions of symbols:

M=body mass
F=total body fat
A=total body water
E=extracellular fluid
I=intracellular water
B=bone mineral
C=cell mass
S=cell solids

Definitions of relationships:

$M = F + A + S + B$
$A = E + I$; $I = A - E$

In conditions where the actual values vary over a certain range the use of assumed (average) biological constants is an expedient which introduces an error into the estimation equations. This error can be reduced or eliminated only by developing independent methods for the evaluation of S and B. What are the quantitative assumptions used in the estimation of these components in the present system and what are the standard errors of the coefficients in equation (5*a*) table 11?

TABLE 11

HYDROMETRIC ANALYSIS: QUANTITATIVE ASSUMPTIONS AND COMPUTATIONAL FORMULAE

Computation of cell solids: $S=C-I$

$$I=0.70\ C;\ \text{then}\ C=I/0.70=1.429\ I$$
$$S=0.429\ (A-E)$$

Computation of bone minerals: $B=k'C$ (where $C=1.429\ (A-E)$)

$$C=M-F-E-B$$

Assuming average values $F=0.14$, $E=.16$, $B=0.06$ and $M=1.00$,

$$C=0.64$$
$$B/C=0.09375=k'$$
$$B=0.134\ (A-E)$$

Computation of body fat: $F=M-A-S-B$

With $M=1$,

$$F=1-A-S-B$$

Substituting for S and B,

$$(5a)\quad F=1-A-0.563\ (A-E)$$

For normally hydrated body ($E=0.16\ M$),

$$F=1-1.563A+0.090$$
$$(6)\quad F=1.090-1.563A$$

The values for S, B and the combined standard error of the coefficient used in calculating F are given in table 12. It is apparent that most of the uncertainty in the estimate of the total fat is related to the hydration of the cells. In the hydrometric method of analysis the effect of the variations in the bone component is small. Using the average (reference) man's values of $A=0.61$ and $E=16$, the ± 1 *S. E.* range of the estimated fat values calculated from equation (7), table 12, would be about 11 and 16 percent of the body weight, respectively.

TABLE 12

HYDROMETRIC ANALYSIS:

STANDARD ERRORS OF THE COEFFICIENTS INVOLVED IN THE COMPUTATION OF S, B, AND F

Cell solids: $S=k_S\ (A-E)$

For the assumed (average) concentration of water in cell mass, 70 percent,

$$k_S=0.429$$

For the one-standard deviation range of ±2.5 percent around the mean (with limits of 67.5 and 72.5 percent) $k_S=0.481$ and 0.379, yielding.

$$S.E.\ of\ k_S=\pm 0.051$$
$$S=(0.429\pm 0.051)\ (A-E)$$

Bone minerals: $B=k_B\ (A-E)$

For the assumed (average) value of $B=0.060\ M$

$$k_B=0.134$$

For the one-standard deviation range of ±0.005 around the mean (with limits of 0.065 and 0.075) $k_B=0.145$ and 0.124, yielding

$$S.E.\ of\ k_B=\pm 0.010$$
$$B=(0.134\pm 0.010)\ (A-E)$$

Total body fat: $F=M-A-S-B$

Combining the coefficients and standard errors for S and B, (5*a*) yields

$$(7)\qquad F=1-A-(0.563\pm 0.052)\ (A-E)$$

In actual analyses the effects of variations in the absolute values of A and E (when obtained by different solutes) and the intraindividual variability (error of measurement) would be added to the fairly large uncertainty resulting from the interindividual differences in the assumed biological constants.

Table 13 presents the data for the conversion of the hydrometric standard into the densitometric equivalent. The value of bone mineral has been taken from the literature. The density of human fat at body temperature has been determined empirically (*11*). The density of the extracellular fluid corresponds to a protein concentration of about 0.5 gm./100 ml. which is the lower limit reported for interstitial fluid. In the computations presented in table 13 the plasma proteins (about 0.3 percent of the body weight) may be thought of as being combined with the cells. Had the plasma proteins been considered as a part of the extracellular fluid, the density of this compartment would have risen to about 1.006 and the density of the cells would have decreased by about 0.001.

TABLE 13

CONVERSION OF THE HYDROMETRIC INTO A DENSITOMETRIC STANDARD OF REFERENCE

($D=M/V;\ V=M/D$)

Component	Density (gm./cc.)	Mass [1] (percent of weight)	Volume [2] (relative)
Bone mineral	3. 0	6	2. 00
Fat	. 9007	14	15. 54
Extracellular fluid	1. 002	16	15. 97
Cells	(1. 0566)	64	(60. 57)
Total body	1. 0629	100	94. 08

[1] kg./100 kg. or gm./100 gm.
[2] dm³/100 kg. or cc./100 gm.

Anthropometric Analysis

Data needed for the estimation of the fat content from skinfold measurements have been reported (*8*). The equations were recalculated in terms of density $D=Sp.\ gr.\times D^{H^2o}$ in the tank; for 35° to 36° C., D^{H^2o} 0.994) and are given in tables 14 and 15. The skinfold thicknesses were determined by calipers with a very small contact surface and high pressure (about 35 gm./mm²) which increased somewhat when the opening of the calipers increased. Further limitation of this particular study is given by the fact that an average correction rather than an individually determined value of residual air has been used. This has decreased, to some extent, the coefficients of correlation between the skinfolds and the specific gravity. Nevertheless, the coefficient of multiple correlation was fairly high for the younger men ($R=0.876$ using all six variables, $R=0.871$ using the selected three variables; for the older men the respective values of R were 0.744 and 0.743).

TABLE 14

EQUATIONS FOR PREDICTING BODY DENSITY ($\hat{Y}=a+bX$) FROM SINGLE SKINFOLDS, IN MM., AND RELATIVE BODY WEIGHT

	Younger men ($N=116$, mean age=22 years)	Older men ($N=214$, mean age=49 years)
1. Skinfolds, abdomen	$1.0990-0.00139\ X_1$	$1.0778-0.00085\ X_1$
2. Skinfolds, chest	$1.0978-0.00158\ X_2$	$1.0805-0.00103\ X_2$
3. Skinfolds, back	$1,1006-0.00176\ X_3$	$1.0786-0.00114\ X_3$
4. Skinfolds, upper arm	$1.1028-0.00230\ X_4$	$1.0819-0.00183\ X_4$
5. Skinfolds, thigh	$1.1049-0.00319\ X_5$	$1.0784-0.00216\ X_5$
6. Relative body weight	$1.1518-0.00078\ X_6$	$1.1161-0.00060\ X_6$

TABLE 15

EQUATIONS FOR PREDICTING BODY DENSITY ($\hat{Y}$) FROM COMBINED SKINFOLDS, IN mm., AND RELATIVE BODY WEIGHT

Using all variables

For the younger men,
$\hat{Y}=1.1058$
$-0.00029\ X_1$
$-0.00066\ X_2$
$+0.00018\ X_3$
$-0.00071\ X_4$
$-0.00037\ X_5$
$-0.00012\ X_6$

For the older men,
$\hat{Y}=1.0901$
$+0.00004\ X_1$
$-0.00042\ X_2$
$-0.00032\ X_3$
$-0.00051\ X_4$
$-0.00025\ X_5$
$-0.00016\ X_6$

Using selected variables

For the younger men,
$\hat{Y}=1.0951$
$-0.00028\ X_1$
$-0.00073\ X_2$
$-0.00088\ X_4$

For the older men,
$\hat{Y}=1.0901$
$-0.00039\ X_2$
$-0.00031\ X_3$
$-0.00059\ X_4$
$-0.00017\ X_6$

This procedure has been devised primarily for work in the field where the more elaborate methods are not applicable, especially when one deals with large numbers of subjects. Nevertheless, it was found useful also in laboratory investigations (*5*).

Problems Needing Further Research

Any single-handed attempt to outline systematic research in the field of methods for the study of body composition would be presumptuous. However, a review of some of the problems that demand further or fresh investigation may be useful.

There is a definite need for additional information obtained by direct chemical analysis of cadavers, preferably combined with the determination of the density of the body as a whole and of its principal, anatomically separable parts. Body dimensions, especially the bony diameters, should also be included. While we are grateful for the labors of the workers at the University of Illinois (Mitchell *et al.* (*22*); Forbes *et al.* (*12*)) and the Department of Experimental Medicine, University of Cambridge (Widdowson *et al.* (*29*)), the data needed for increasing the fund of basic knowledge relevant to an indirect evaluation of body composition require less comprehensive analyses than had been undertaken by these investigators. An important contribution would be made by increasing the number of cadavers analyzed, with concern for the relative clinical normality of the subjects, but reducing the load of work by limiting the analysis to the determination of water (by desiccation), fat (ether extract), and bone minerals (ash), with the cell solids obtained by difference ($S=M-A-F-B$). Data on cadavers closely similar to the normal young man, used as the reference point in the Minnesota system of analysis, would be particularly useful.

Preliminary reports have been published about the determination of body volume by air displacement, either by changing the pressure in the enclosed space containing the subject (*28*) or by the dilution of a foreign gas (helium in Siri's report (*27*)). More detailed description of the procedures, their technical difficulties, reliability of repeated measurements, and their validity as compared with underwater weighing will be of considerable interest.

As indicated earlier, an attempt was made to provide a common frame of reference for the three approaches described here. This has been done on the basis of empirical data (conversion of skinfold thicknesses into body density), and partly on the basis of theoretical considerations (conversion of hydrometric analysis into densitometry). Simultaneous application of the hydrometric and densitometric methods, as has been carried out for the simpler estimation of body fat from total body water and specific gravity of the body by Messinger and Steele (*20*) and Osserman *et al.* (*23*), should reduce some of the present uncertainties in the conversion factors.

Finally, it is desirable to provide additional data for predicting body density on the basis of the minimum number of skinfold measurements made at locations recommended for general use (over the triceps, at waist, below scapula) using calipers with a constant tension of 10 gm./mm^2.

Acknowledgment

It is a pure formality that the paper bears the signature of one author. Dr. Ancel Keys, director of the Laboratory of Physiological Hygiene, had a large share in the development of the ideas and the arithmetical gymnastics. Of the other members of the Laboratory at least Dr. Joseph T. Anderson and Mr. Walter Carlson should be mentioned by name as having contributed brain and manpower to the studies on body composition.

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Body Fluid Compartments and Indices of Body Composition

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The concept of a metabolically active, fat-free mass (*FFM*) of constant composition is an attractive thesis to those interested in assessing nutritional status from total body composition (*3*). It has stimulated several physiologically rational approaches for assessing body composition in man (*9*). One of these approaches is by water (*11*). If body water is a constant fraction of the *FFM*, it should be possible to determine the weight of the *FFM* simply from measurement of total body of water: $FFM = \frac{\text{total body water}}{k}$ where k = the fraction of the *FFM* represented by total body water. The body fat is then simply the difference between total body weight and *FFM*. Various attempts have been made to measure k and establish it as a physiological constant. Thus, Rathbun and Pace (*12*) analyzed the bodies of 50 eviscerated guinea pigs for water, fat, and nitrogen, and calculated that total water was 72.4 percent ± 2.11 of the fat-free tissue. Similar direct analyses have been reported on very few cadavers. Keys and Brožek (*9*) have summarized this work, and list water as 73.2 percent and 68.4 percent of *FFM* in the only two human cadavers that could be considered "normal."

Attempts have been made to measure the percent of water in the *FFM* of living persons by indirect means. Osserman *et al.* (*10*) measured total body water in 81 subjects, using antipyrine (*13*). They estimated *FFM* from body specific gravity (*4*), using the equation of Rathbun and Pace (*12*):

$$\text{Percent body fat} = 100\left(\frac{5.548}{sp.\ gr.} - 5.044\right).$$

They reported that antipyrine space constitutes 71.8 percent of the *FFM* (range 66.3–79 percent).

Almost all efforts to assess body composition from fluid compartments have been directed at total body water. However, Crandall and Anderson (*5*) early suggested that thiocyanate space was correlated with metabolic activity. Dahlstrom (*6*) found close correlation between "extracellular" fluid (thiocyanate space) and BMR regardless of age or sex. Recent work from this laboratory emphasizes the possible usefulness of *SCN* space as an index of body composition. Thus, it was found that this space has a significantly higher coefficient of correlation with *FFM* (estimated by the skinfold method) than with body weight (*1*). In a further study (*14*) it was found that *SCN* space correlated better than all other fluid compartments with basal metabolism in a series of 17 healthy young soldiers. The impact of the extracellular fluid on the prediction of BMR was twice that of the intracellular fluid. If basal metabolism may be considered a measure of active metabolic mass, these findings may provide useful clues for assessment of body composition from extracellular fluid volume.

During the past 2 years we have measured antipyrine (*AP*), thiocyanate (*SCN*), and T–1824 spaces in 54 healthy young soldiers. The data are being prepared for publication (*2*). All three spaces were measured simultaneously by a single injection. In addition, percent of body fat was determined from skinfold thickness (*9*). The data are summarized in table 1.

The average values for plasma volume and antipyrine space agree with those of other workers. The *SCN* space is higher than that reported by Henschel *et al.* (*8*). However, they allowed less than 1 hour for diffusion of *SCN*, whereas we allowed 2 hours on the basis of a study of the kinetics of diffusion (*1*). The coefficients of variation are smallest for all three compartments when expressed as percent of *FFM* (table 1), indicating that the skinfold technique at least partially removes a source of variability, presumably fat. The reduction in variability was small; this probably reflects the homogeneity of the sample with respect to age and fat, since the ages covered only a single decade (19–29 yr.), and the range of fat was relatively narrow.

From the data in table 1, we calculated the *FFM* of each subject by four methods:

1. $FFM = \frac{AP \text{ space}}{0.718}$ (*10*)
2. $FFM =$ Total body wt. (100—percent fat from skinfolds).
3. Percent fat $= 109.0 - 1.563\ AP$ (equation No. 40 from (*9*)).
4. Percent fat $= 100.0 - 1.563\ AP + 0.349\ SCN$ (equation No. 38 from (*9*)).

In methods No. 3 and No. 4 above, *AP* and *SCN* are expressed as percent of body weight, and the fat was subtracted from body weight to obtain *FFM*. These calculations are summarized in table 2.

The mean *FFM* from skinfolds was 62.32 kg. as compared with 55.08, 56.25, and 56.18 by the other three methods. This difference was highly significant. The close agreement among methods 1, 3, and 4 is not surprising, since all three methods assume total water to

TABLE 1

BODY FLUID COMPARTMENTS OF HEALTHY YOUNG SOLDIERS (54 men; 19–29 yr.)

	Weight	Fat-free Mass ("FFM") from skin-folds [1]	Plasma volume (T–1824)			"Extracellular" fluid (thiocyanate)			Total body water (antipyrine)		
			Liters	Percent body weight	Percent FFM	Liters	Percent body weight	Percent FFM	Liters	Percent body weight	Percent FFM
Mean	67. 83	62. 32	2. 979	4. 41	4. 79	17. 62	26. 08	28. 31	39. 54	58. 81	63. 63
S. D	8. 90	6. 80	. 401	. 55	. 52	2. 63	3. 07	3. 03	4. 15	6. 36	5. 60
Coefficient of variation	13. 1	10. 9	13. 5	12. 4	10. 9	14. 9	11. 8	10. 7	10. 5	10. 8	8. 8

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[1] Average percent fat = 7.79±3.30 percent.

TABLE 2

FAT-FREE MASS OF 54 MEN ESTIMATED BY VARIOUS METHODS

	1	2	3	4	Coefficients of Correlation
	AP space [1]	Skinfold	Keys and Brožek [2]	Keys and Brožek [3]	
FFM (kg.)	55.08	62.32	56.25	56.18	$r_{12}=0.64$ $r_{23}=0.64$ $r_{24}=0.61$ $r_{34}=0.99$
S. D.	5.78	6.80	6.74	6.46	
C. V.	10.5	10.9	12.0	11.5	

[1] $FFM=\frac{AP\text{ space (liters)}}{0.718}$

[2] From equation No. 40: percent F=109.0−1.563 *AP*.

[3] From equation No. 38: percent F=100.0−1.563 *AP*+0.349 *SCN*.

comprise approximately 70 percent of the *FFM*. Although the *FFM* from skinfolds correlated significantly with those from other methods, the low correlation coefficients (r=0.61 to 0.64) indicate a low resolving power for prediction in individual cases. Although all these calculations undoubtedly account for some of the fat, the discrepancy between the *FFM* from skinfolds and that from body water requires clarification, and suggests the following possibilities: (*a*) Total body water is appreciably less than 70 percent of the *FFM*; (*b*) skinfolds underestimate body fat content; (*c*) Our measurement of skinfold thicknesses is systematically different from Brožek's; (*d*) A combination of (*a*), (*b*), and (*c*).

It is possible that total body water is not so constantly related to *FFM* as is generally assumed (*7*), and that some fraction of the body water may provide a better measure of *FFM*. Our earlier studies indicated that *SCN* space may be a better reference standard than total body water (*1, 14*). To test this further we are in the process of analyzing the data on 54 men by partial correlation techniques. The primary (zero order) correlation coefficients are summarized in table 3.

TABLE 3

PRIMARY CORRELATION COEFFICIENTS (r) BETWEEN BODY FLUID COMPARTMENTS IN 54 SUBJECTS [1]

Y / X	6 ISF	5 ICF	4 AP	3 SCN	2 PV
1. FFM	0.62	0.32	0.64	0.68	0.58
2. PV	.34	.14	.39	.50	-------
3. SCN	.98	−.02	.51	-------	-------
4. AP	.47	.85	-------	-------	-------
5. ICF	−.05	-------	-------	-------	-------

[1] 1=FFM (skinfolds).
2=PV (T-1824).
3=SCN space.
4=AP space.
5=ICF (AP-SCN).
6=ISF (SCN-PV).

Thiocyanate space had the highest correlation with *FFM* ($r=0.68$). The intracellular fluid volume (*ICF*) had the lowest correlation with *FFM* ($r=0.32$), despite the high correlation between *AP* and *ICF* ($r=0.85$). The correlation between *SCN* and *ICF* was negligible ($r=-0.02$). This interesting finding may be merely a reflection of cumulative experimental errors, since the *ICF* includes the errors of both *AP* and *SCN* determinations; the homogeneity of this series would favor such masking. On the other hand, the lack of correlation could indicate that the "stat" mechanisms for these two compartments (*SCN* and *ICF*) operate independently of each other to a certain degree. It has been shown that the impacts of these two compartments on basal metabolism are independent of one another (*14*). Partial (first order) correlations of the present study reveal a similar independence in their respective correlations with *FFM* (*2*).

General Comment

The concept of a fat-free mass of constant water content appears to have physiologic rationale. However, since total body water represents two major *milieux interieures* (extra- and intracellular water), it is possible that one of these provides a better reference standard for body composition. Data presented here indicate that thiocyanate space is as good or better than total body water and superior to intracellular fluid for this purpose. The relatively low correlation coefficients found indicate that simple measurement of any fluid compartment has poor prediction value for *FFM*.

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Relation of Body Composition to Metabolic Activities[1]

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The previous speakers have considered the importance of *in vivo* quantitation of body composition to the nutritionist and have discussed the various techniques by which this composition might be estimated. My remarks will be concerned with some facts and speculations regarding the theoretical and practical relationships of body composition and metabolic activities.

Basal oxygen consumption has generally been considered the best available index of general metabolic processes. Traditionally it is expressed per unit of surface area. This use of surface area has recently been challenged (*1*, *2*, *3*, *6*, *9*, *10*, *11*, *12*, *18*). The basic question involved is this: "Does each mass of metabolically active tissue utilize oxygen at a fairly uniform basal rate regardless of the relative size of an individual within the species? Or does the overall size as represented by surface area monitor the rate of utilization within each constituent metabolically active mass?" Surface area does show a general correlation with basal metabolism when comparing all mammals from the mouse to the elephant (*10*). This relationship apparently is due to heat dissipating needs in the maintenance of a constant body temperature. Within a species, however, the vascular and respiratory mechanisms can readily compensate for widely varying needs for heat exchange. Though a good correlation may be found between surface area and oxygen consumption within a species, this does not establish a prime relationship. Many spurious correlations related to overall body size may be found. In this regard it is worth reemphasizing the fact that the commonly employed formula of DuBois and DuBois (*5*) estimates the surface area from height and weight alone. It was derived in a rather empiric fashion to fit the actual surface area as measured by body molds in only 6 men and 3 women. By analogy with specific internally regulated biochemical systems, it appears more probable that in the basal state each unit of active mass is reacting at a rate independent of the total organismal size. This problem has been considered by several groups, most of whom are represented on this panel. Some of the evidence has been independently reviewed by Keys and Brožek (*9*) and by Behnke (*1*).

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[1] The data for this paper were obtained at the Army Medical Nutrition Laboratory during the time of the author's services as medical officer.

It may be summed up as follows: (*a*) *Changing nutritional states in individuals.* In starvation and rehabilitation the basal oxygen consumption of individuals remains more nearly constant when calculated on the basis of active tissue mass or fat-free mass than on the basis of body weight or surface area. (*b*) *Comparison of different age groups.* Older men of the same average height, weight, and surface area as a younger group showed lower rates of basal oxygen consumption. The older men had a higher percentage of body fat, and when the metabolic rate was corrected for active tissue mass the rates were almost the same. (*c*) *Comparison of the two sexes.* The apparently lower rate of metabolism of females when computed on the basis of calculated surface area becomes essentially the same as for males when based on metabolically active mass. (*d*) *Comparison of individuals in the same age-sex group but with an array of body types.* Basal oxygen consumption in milliliters per minute has been correlated with various body measurements. Highly significant correlations are found with height, weight, surface area, and lean body mass, as well as other body measurements (*2, 3*). This is not unexpected, as all of these measures are closely related to each other in most individuals. Miller and Blythe in 48 subjects had a correlation coefficient of 0.84 for oxygen consumption with surface area and of 0.92 with lean body mass (*12*). These values are compared with others in table 1. In 22 young men we noted corresponding coefficients of 0.80 and 0.88 (*2, 3*). In neither of these first two studies was the difference in coefficients statistically significant for the number of

TABLE 1

COMPARATIVE CORRELATIONS OF BASAL OXYGEN CONSUMPTION WITH VARIOUS BODY MEASUREMENTS

Author	Number of subjects	Method of estimating active protoplasmic mass	Correlation coefficient with basal oxygen consumption		
			←	Surface area	Body weight
Miller and Blythe (*12*).	48	Specific gravity-LBM.	0. 92	0. 84	0. 85
Best *et al.* (*2, 3*)	22	Skinfolds-LBM	. 88	. 80	. 8–
Steele *et al.* (*17*)	82	"Antipyrine space"	. 74	. 75	
Wedgwood *et al.* (*18*).	17	"Antipyrine space"	. 76	. 74	. 68
Garn *et al.* (*6*)	49 (M)	"Thiocyanate space".	. 80	. 74	. 68
		Leg muscle width by X-ray in children.	. 82	. 83	. 8
	46 (F)		. 73	. 65	. 68

subjects included. Similar correlations have been obtained using other estimates of active protoplasmic mass. Steele and associates found practically equal correlations of basal oxygen consumption with total body water by antipyrine determination and surface area (*17*). Wedgwood *et al.* had similar results (*18*). These authors did show a superior correlation of "thiocyanate space" with oxygen consumption. They noted that there is an appreciable correlation of thiocyanate space with surface area. When thiocyanate space is held constant, the partial correlation of oxygen consumption with surface area becomes negligible. Garn and associates noted an equal or superior correlation of oxygen consumption with radiographically determined calf muscle width as compared to surface area or body weight (*6*).

The total evidence, though still not conclusive, strongly suggests that metabolism is closely related to the active protoplasmic mass regardless of other parameters of total size.

This leads to an important corollary: *The basal oxygen consumption of normal individuals should be used as the standard for evaluating various physical estimates of the active protoplasmic mass.* For practical reasons it would be desirable to have a method for the prediction of active protoplasmic mass from simple measurements such as height, weight, age, and sex. In deriving suitable equations for this purpose, the basal oxygen consumption of normal individuals of all ages, both sexes, and all body configurations should be utilized. The proper formulae may then be derived by the logical application of statistical techniques. The factor necessary to convert predicted rate of oxygen consumption into kilograms of active protoplasmic mass is at present not known. For the present, one must be satisfied with this index alone. We have done a pilot study of this nature on the previously mentioned 22 young men (*2*). It is apparent that within a group having a spectrum of leanness-fatness configurations, height and weight alone are inadequate for the estimation of lean body mass. Two individuals, one heavy-boned and muscular, the other light-framed and obese, might have identical measurements of height and weight. Some independent measure of relative obesity is necessary for the accurate prediction of active protoplasmic mass. It would be desirable to have such a measurement which could be taken by a relatively inexperienced technician or physician with satisfactory accuracy and with minimal discomfort or embarrassment to the patient. Possibilities include abdominal girth, posterior brachial skinfold thickness by caliper measure, or a combination of several caliper measurements. In our study, abdominal circumference at the umbilicus following normal expiration was used for this purpose.

A statistically derived prediction equation should fulfill the following criteria: (*a*) It should predict the oxygen consumption with greater accuracy in normal subjects than do other proposed equations. (*b*) It should be dimensionally consistent (e. g., as the estimate is of active protoplasmic *mass*, a formula should give the equivalent of a linear measurement to the third power). (*c*) It should have geometric, anatomic, and/or physiologic meaning, if possible. Several types of formulae were tested, and the one giving the best prediction in a single, simple equation was:

(1) $\text{ml. } O_2/\text{min.} = 4.51 \times \text{kg. body wt.} - 165 \text{ (meters abd. circ.)}^3 + 2.56$

Height was found unnecessary for this equation. It is our hope that when enough raw data of this type are analyzed, a single equation or series of equations may be derived to express the normal basal oxygen consumption and active protoplasmic mass in terms of sex, age, weight and/or height, and a single index of obesity. Except for an index of obesity this is essentially the approach of Harris and Benedict (*7*). These formulae could then be converted into nomograms or slide rules for ready clinical and experimental reference. The *BMR* in individual cases would then be expressed directly as a percentage of the theoretical oxygen consumption.

There is also suggestive evidence that other measures such as cardiac output, respiratory minute volume, renal clearance, and urinary creatinine excretion might better be expressed on the basis of active protoplasmic mass. The creatinine excretion rate under standard conditions is presumedly related to the total muscle mass, the largest single component of the active protoplasmic mass. It should therefore show a superior correlation with measures closely related to active protoplasmic mass. In our series of 22 men we found correlations of creatinine excretions to be 0.67 with basal oxygen consumption, 0.61 with lean body mass, 0.59 with surface area, and 0.57 with body weight (*2, 3*). These correlations are not significantly different from one another on the basis of the number of men included. Miller and Blythe (*11*) have also shown a close agreement between creatinine excretion and lean body mass.

My comments so far have been concerned with the body metabolism in the basal state. On speculation it appears equally probable that the body composition is of importance in the expenditure of energy in active states and in relation to weight changes during periods of energy imbalance. The literature gives only crude average values for the energy cost of various activities. There are no good data relating this cost to body composition. I should like to make the armchair postulates that two extremes of energy expenditure might be recognized: (*a*) Relatively quiet activities in which the caloric expenditure is closely related to the lean body mass (as a measurable estimate of active protoplasmic mass). This would include sleeping, lying quietly awake, sitting motionless, and standing still. (*b*) Relatively vigorous activities in which the expenditure is related to the work accomplished in foot-pounds or dyne-centimeters. In such activities the work would be related to the load or total body weight (i. e., lean body mass plus weight of fat). Examples would include climbing, jumping, and performing certain calisthenics. Between these extremes should be a series of activities in which variable expenditure rates might be found in relation to lean body mass and to the additional load of body fat. At each time of the day, t, the rate of expenditure of the current activity related to lean body mass can be taken as x, and the additional amount related to excess fat as y. Then, for a given day, the energy expenditure would be:

$$\text{(2)} \qquad \text{Cal. expenditure} = LBM \int_{24\text{ hr.}}^{O} \times\, dt + BF \int_{24\text{ hr.}}^{O} y\, dt$$

in which LBM represents lean body mass, and BF represents the weight of total body fat. In the course of another experiment (*16*), we observed 81 soldiers[2] during two 3-week periods in which they engaged in a vigorous and relatively uniform, and constant activity program as monitored by close observation and analysis of activity records. During the first period their average measured caloric intake was 3453.4 cal./d., and they lost an average of 0.027 kg./d. During the second period their average measured caloric intake was 2263.5 cal./d., and they lost an average of 0.190 kg./d. Assuming a uniform relation of weight loss to caloric deficit the following formulae may be constructed:

$$\text{(3)} \qquad 0.027 \text{ kg./d.} = k_1 \ (C_a + SDA - 3454.4 \text{ cal./d.})$$

$$\text{(4)} \qquad 0.190 \text{ kg./d.} = k_1 \ (C_a + SDA - 2263.5 \text{ cal./d.})$$

Specific dynamic action, SDA, was calculated to be 7.3 percent of the first caloric intake and 7 percent of the second. C_a is the caloric expenditure related to activity states. By solution of simultaneous equations one obtains a value of 0.000149 kg./cal. for k_1, and 3385 cal./d. for expenditure exclusive of SDA. The former value is equivalent to 6.74 cal./gr. weight change, which is within the range of previous estimates (*8, 9, 16*).

During the second restricted caloric period lean body weight and body fat as estimated from skinfold measurements and total body weights at the beginning of the period were recorded (*4, 14*). The inter-individual variations in food intake were slight enough (s. d.=54.7 cal./d.) that we considered intake as uniform. The format of equations 3 and 4 was combined with the spirit of equation 2 to give:

$$\text{(5)} \quad \text{kg./d. wt. loss} = k_1[(k_2 LBM + k_3 BF + k_4) + SDA - \text{cal./d. intake}]$$

Using the value of k_1 obtained above plus the measured data from each man, the following multiple regression equation was derived according to standard statistical techniques (*15*).

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[2] Six of the original 87 subjects were excluded due to interfering periods of illness or injury.

$$\text{(6)}\quad \text{kg./d.}=0.000149\,[(29.1\,LBM+35.0BF+1317)+SDA-\text{cal./d. intake}]$$

The three terms in parentheses, representing the daily expenditure related to activity states, are to be compared with predicted values had the men remained at all times in a basal state (*2*).

$$\text{(7)}\quad \text{cal./d. expend.}=22.4\,LBM+117.5$$

or 1616 cal./d. for these men of average LBM, 66.9 kg. Comparison of equations 6 and 7 shows increased factors for all components of expenditure during this particular vigorous program. The particular values of k_2, k_3, and k_4 of equation 5 which are found in number 6 cannot be applied outside of the conditions found in this particular study. The fact that values of proper sign and reasonable magnitude may be derived for such constants from the impartial analysis of carefully gathered field data, however, lend encouragement to the theories embodied in equation 2. The relation of body composition to the energy cost of standardized activities is a subject deserving of further intensive laboratory investigation.

In studying the programs of caloric over- and under-nutrition, data must be obtained on the following aspects with relation to body composition: (*a*) Actual caloric intake. The study of Peckos (*13*), though lacking in precision and completeness, is a start in this direction. (*b*) The actual utilization of calories ingested. (*c*) Detailed account of daily activities. (*d*) Relative conservation or extravagance of energy in performance of daily tasks. As a casual observation, one is sometimes impressed with the fact that the obese individual does things the lazy, phlegmatic, conservative way; while the lean man is frequently hyperkinetic, fidgets, and injects more body motion than necessary into his daily activities. In relation to the same field experiment which I have here discussed (*16*), Dr. Grossman has outlined this afternoon a slightly different method of analysis. On 35 subjects showing less than 0.4 kg. weight change during a particular period he has found the following regression equation:

$$\text{(8)}\quad \text{cal./d. intake}=22.8\text{ body wt.}-55.2\text{ percent}+2316.2$$

This is to be compared with the three terms in the parentheses of equation 6. k_2 and k_3 of that equation are so nearly the same that a single constant for body weight (lean body mass plus fat) should be almost as good in this instance. The interesting feature is the decreasing caloric expenditure with increasing relative obesity despite a uniform activity program. This finding may lend some support to the concept that efficiency in performing particular tasks is related to the leanness-fatness configuration. (*e*) The actual caloric costs of standardized activities as discussed above. (*f*) The weight equivalent of caloric imbalance. It seems reasonable that the composition of tissue accounting for weight gain or loss in the lean may be different from that in the obese. If this is so, the caloric equivalent of weight change should vary according to the initial body composition.

In summary, there is an increasing body of evidence which tends to indicate that active tissue mass or some closely related measure such as lean body mass might be superior to surface area for the estimation of basal oxygen consumption. The implication is that within a species there is a characteristic metabolic rate for each average mass of active tissue.

It is suggested that the basal oxygen consumption of normal individuals be used as the reference standard for evaluating other estimates of active protoplasmic mass. Other physiologic and metabolic measurements including creatinine excretion appear closely related to this mass.

It is postulated that energy expenditure is variably related to lean body mass and to the additional load of body fat depending on the type of activity. Statistical data from a field study of 81 men during two 3-week periods are present. These data are compatible with such a postulate. Additional theoretical considerations relating body composition to energy balance are discussed.

As simple, reliable methods of the quantification of body composition gain general acceptance, they should lead to more precise establishment of physiological norms and to more adequate evaluation of nutritional requirements.

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MODERATOR GROSSMAN:

I will ask the other members of the panel to join me at the so-called round table.

I would like first to ask the members of the panel to discuss each other's work, and I believe that it would be appropriate at this time to ask Captain Behnke to comment on the presentations that he has heard.

Body Composition. I. *In Vivo* Estimation of Fat and Water Content from Specific Gravity (Density) Determinations

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The specific gravity (sp. gr.) or density (*D*) of the body as a whole (*W*) is an index of its gross composition. In 1942, Behnke, Feen, and Welham (*4*) examined the premise that body fat because of its relatively low density was most likely the chief variable influencing the density of the body as a whole. They developed a procedure which made possible for the first time an accurate *in vivo* estimate of the body fat fraction which in turn permitted an estimate to be made of the size of the relatively homogeneous lean body mass. The background for this procedure consists in the numerous chemical analyses of tissues and carcasses of various animal species during the past 4 decades by Murray, Moulton, Brody (*7*, *16*, *17*), and other investigators. Their data demonstrated a remarkable uniformity of the mean percentages of various structural elements that make up gross composition of the fat-free adult mammalian body.

Subsequently, experimental data on species as different as guinea pigs and cattle have confirmed the basic premise *that the percentage of fat relative to gross body weight in adult mammals is inversely proportional to the specific gravity or density of the body as a whole.* In addition, determinations of total body water (by means of antipyrine, deuterium, tritium techniques) have provided another approach to an understanding of gross body composition. In what follows we shall cite a number of principal investigations centering in densimetry and body water analyses in order to evaluate the accuracy of prediction of the percentages of fat and water in body weights of given individuals.

Part II of these comments will deal with early studies in which measurements of gases dissolved in body tissues, i. e., nitrogen and helium, permit a partition of the body into its lipid and aqueous components. This principle has recently been applied in a conclusive manner by Lesser, Blumberg, and Steele (*14*) who measured body fat in rats, using cyclopropane.

Definitions

(*a*) *Specific gravity* refers to the weight of a body in air divided by its loss of weight (corrected for air in the lungs) in water. (*b*) *Fat.* The distinction between *pure fat* (F), as extracted by ether and other solvents, and *tissue lost or gained* ($F + s$) by healthy individuals under controlled conditions of diet which tend to maintain nitrogen balance is important as emphasized by Keys and Brozek (*12*). The existence of the $F + s$ components revealed by the densimetric studies (density 0.92 to 0.94) has been referred to in our previous papers as "tissue gained or lost," "excess fat," "adipose tissue," and simply, fat. The F or ether extract substance is the major fraction; the s component appears to be a small, *constant* percentage of $F + s$, essentially water and dry matter which is gained or lost by the body together with gain or loss of fat (F). Although the precise nature of the s component is not known, it does not apparently change the relative proportion of water, protein, and mineral in the fat-free body. Tentatively, it appears to be inseparable from the lean body mass (LBM) and in a sense, is "growth substance" and not associated with abnormal hydration.

Procedure

For the determination of specific gravity, the essential measurement, based on Archimedes' principle, is the quantity of water displaced by the immersed body which can be ascertained conveniently by the method of hydrostatic weighing. The difference between the weight in air and the weight in water, divided by the density of the water, measures the volume of the body. It has proved to be sufficiently accurate for our purpose only to divide weight in air by the weight lost in water to obtain specific gravity without converting the buoyant force into its volume equivalent and expressing the value as density. The comfortable water temperature for quiescent individuals is not more than a degree or two from 31° to 32° C. and this was the approximate range of temperature in all human immersion experiments. When isolated tissues or carcasses were examined, the water temperature was about 20° to 25° C. The percentage of fat is approximately the same when it is computed from either a "specific gravity" or a "density" formula.

Two weights recorded when the body is immersed in water serve to ensure the accuracy of the procedure, one taken at the completion of maximal inspiration, and the other at the end of maximal expiration. The difference between these weights converted to the volume equivalent and corrected for the effect of mean hydrostatic pressure on thoracic volume, is a measure of vital capacity comparable to that obtained by standard spirometry.

Sources of error. The chief variables affecting accuracy of replicate analyses center in the determination of residual air volume and in the presence of gas in the alimentary tract. Despite these difficulties, it is possible, with the same attention to detail required in any quantitative procedure, to obtain replicate values on the same individual which are within the range of 0.004 specific gravity units, represented by a standard deviation of ± 0.0006 specific gravity units.

Experimental Data

It was found that in a group of 175 Navy men (average age, 29) the specific gravity values ranged from 1.021 for the most obese individual to 1.097 for the leanest man. There was no doubt from gross inspection of the Naval subjects that low values of specific gravity were generally associated with overt obesity, and high specific gravity values with leanness. Then, by means of a single crucial experiment extending over a period of 7 months, it was found that the weight loss of 19.5 pounds in an individual on a reduced caloric but high-protein diet, was inversely proportional to the specific gravity of the body as a whole. From several specific gravity values recorded during the period of weight loss, it was computed that the density of the "lost tissue" was of the order of 0.93 to 0.94, i. e., the density of adipose tissue.

From two basic assumptions, namely, that fat was the chief variable altering specific gravity, and that the lean body mass was of homeostatic constancy approximating a specific gravity value of 1.100, it could be computed that a specific gravity value for the body as a whole of 1.082 indicated about 10 percent fat (termed "essential" in the original report of Behnke, Feen, and Welham (*4*)) and that a value of 1.035 indicated about 36.5 percent total body fat of which 26.5 percent was regarded as "excess."

The inverse relationship between the fat fraction of body weight and specific gravity, initially regarded as linear, was later correctly formulated by Morales *et al.* (*15*) as,

$$\frac{F}{W}=\frac{\dfrac{1}{D\text{ body}}-\dfrac{1}{D\text{ lean or fat f.b.}}}{\dfrac{1}{D\text{ fat}}-\dfrac{1}{D\text{ lean or fat f.b.}}} \qquad (1)$$

This basic formulation which implies a rectangular, hyperbolic relationship underlies all subsequent equations, e. g., those of Rathbun and Pace (*20*), which have been so helpful for the calculation of the percentage of body fat from specific gravity or density values.

The calculations in the original report quoted previously relating specific gravity to specific percentages of adipose tissue ($F+s$) may be fitted into the framework of (1) as follows:

$$(2) \qquad \frac{F+s}{W}=\frac{7.163}{\text{sp. gr.}}-6.620, \qquad \text{sp. gr.}\frac{F+s}{31^\circ\ C.}=.94 \qquad \text{sp. gr.}\frac{LBM}{31^\circ\ C.}=1.082$$

where *LBM* included 10 percent essential fat.

Converting to density, and with reference to the fat-free body, (2) becomes,

$$(3) \qquad \frac{F+s}{W}=\frac{6.451}{D}-5900, \qquad {}^{D}F+s=.933 \qquad {}^{D}\text{fat f. b.}=1.094$$

At this point it may be well to emphasize that equations (2) and (3) represented at the time only a first approximation in the *in vivo* analysis of gross body composition. It was the objective to study systematically the variation of such components as water, mineral, and protein in the *LBM* or fat-free body, and in turn to relate these entities to metabolism. Subsequently, the objective in part has been realized in the investigations of DaCosta and Clayton (*8*), Kraybill *et al.* (*13*), Morales *et al.* (*15*), Pace and Rathbun (*19*), and Rathbun and Pace (*20*) which have clarified the relationship between specific gravity on the one hand, and extracted fat and *in vitro* body water analyses on the other hand in guinea pigs, rats, and cattle (tables 1 and 2).

TABLE 1

PERCENTAGE FAT CALCULATED FROM SPECIFIC GRAVITY COMPARED WITH ANALYSES BY OTHER METHODS

			Standard deviation of difference
Osserman *et al.* (*18*)	Percent fat from specific gravity.	Percent fat from antipyrine space.	±3.26, $r=0.900$
Kraybill *et al.* (*13*)	Percent fat from specific gravity.	Percent separable fat.	±1.59, $r=0.956$
Rathbun and Pace (*20*).	Percent fat from specific gravity.	Percent fat ether extract.	±1.87 [1], $r=0.972$

[1] Standard error of prediction of percent fat (gross weight) from specific gravity.

When Brodie (*6*), Soberman (*22*), and J. Murray Steele (*23*) and their coworkers investigated the feasibility of computing body fat from total body (antipyrine) water determinations, it became possible to compare densimetry values with the major component of the *LBM*. Such studies were extended in man by Osserman *et al.* (*18*), and the procedures applied in animal husbandry (cattle) by Kraybill, Bitter, and Hankins (*13*). In what follows we shall outline the results of these investigations.

TABLE 2

Comparison of Percentages of Total Body Water From Specific Gravity, Antipyrine, and Dessication Procedures

Investigator	Procedure 1	Procedure 2	Results: Mean percent water *LBM* or *FFB*	Results: S. D. of difference between 1 and 2, or correlation
Soberman (*22*) (Several species)	Percent H_2O dessic. 71.3 (66.4–77.0).	Percent H_2O AP 70.9 (62.8–77.8).	71. 1	±2.6, r=0.93
Kraybill *et al.* (*13*) (30 cattle)	Percent H_2O sp. gr	Percent H_2O AP	72. 6±1. 6	±0.284, r=0.984
Pace *et al.* (*19*) (50 guinea pigs)	Percent H_2O dessic	Percent H_2O sp. gr	72. 4±2. 11	r=0.88 [1]
Osserman *et al.* (*15*) (81 men)	Percent H_2O AP	Percent H_2O sp. gr	71. 8±2. 9	±3.26, r=0.90
DaCosta, Clayton (*8*) (458 rats)	Percent H_2O dessic	Percent H_2O sp. gr	Normal diet Restricted diet Return to normal	r=0.83 r= .86 r= .95

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[1] Calculations of Keys and Brožek (*12*).

Morales and his coworkers (*15*) divided the body into a five-phase system (fat, muscle, skin, nerve, and bone) and from a summation of values of these individual tissues of guinea pigs, they obtained a value of 1.099 as the density of the lean body mass. This value was nearly identical with the value (1.098) for the specific gravity, approx. 23°/23° C., of an aliquot sample of whole body guinea pig homogenate (*20*). For man, a rounded value of 1.100 (or approximately the specific gravity value obtained on extremely lean individuals) was ascribed to the *LBM* and 0.918 as the density of fat. From these values the formula of Rathbun and Pace for man may be written as,

$$(4) \qquad \frac{F}{W} = \frac{5.548}{\text{sp. gr. } (31^\circ \text{ C. approx.})} - 5.044, \qquad {}^{[1]}D_F = .918 \quad {}^{[1]}D_{LBM} = 1.100$$

From densimetric determinations and chemical analyses of the carcasses of guinea pigs (*19*, *20*), classical data was provided which not only tended to support the concept of an inverse relationship between the fat fraction of the body mass and specific gravity ($r = -0.97$), but also to elucidate the direct proportionality between specific gravity, and total body water. Thus, "a straight line fit was made by the method of least squares to the experimental values of fat content versus the inverse of specific gravity and the equation

$$(5) \qquad \text{Percent fat} = 100\left(\frac{5.135}{\text{sp. gr.}} - 4.694\right) \qquad {}^{[1]}D_F = .9018 \quad {}^{[1]}D_{\text{fat}}\,\text{f. b.} = 1.0939$$

represents the least squares hyperbola through the data. The correlation coefficient was computed to be -0.972, and the standard error of y on x was 0.0187, or ±1.87 percent fat content."

With reference to the estimation of body fat from the percentage of water in gross weight, the Pace-Rathbun equations are,

$$(6) \qquad \text{Percent of fat} = 100 - \frac{\text{percent water}}{0.732}$$

and

$$(7) \text{ and } \quad \text{Percent water} = 100\left(4.424 - \frac{4.061}{\text{sp. gr.}}\right)$$

Osserman *et al.* (*18*) carried out specific gravity and antipyrine space determinations on 81 men (18 to 46 years). Fat was calculated from equation (4). The correlation between body fat and body water was -0.90; the percentage of fat varied from 0 to 38.4, the mean percentage of water in the *LBM* was 71.8±2.9 percent. The S. D. of the differences between percent F calculated from specific gravity, and percent F from antipyrine space, was 3.25 (table 1).

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[1] Inserted by this author.

Kraybill, Bitter, and Hankins (*13*) obtained excellent agreement between the percentage of separable fat, antipyrine space determinations, and $\frac{\text{sp. gr.}}{20°/20° \text{ C.}}$ in 30 cattle. I have substituted their specific gravity data in the following formula which represents but a slight alteration of the Rathbun-Pace formula (4),

$$(8) \qquad \frac{F}{W} = \frac{5.263}{\text{sp. gr.}} - 4.784, \qquad \begin{matrix} D_F = .910 \\ D_{LBM} = 1.100 \end{matrix}$$

The S. D. of the difference between the percentage of separable fat and the percentage calculated from specific gravity was 1.59, or $\overline{X} = 0.1 \pm 1.59$, $r = -.956$. The correlation between the percentage of water in gross body weight and specific gravity was 0.984. The percentage of water in the *LBM* was 72.6. The S. D. of the differences between percentage of water (determined as antipyrine space) and percentage of water (from specific gravity) was 0.284, or $X = 0.0 \pm 0.284$ (table 2).

Discussion

Accuracy of prediction of F or F + c, and LBM or fat-free body. For precise results the specific gravity technique requires the same meticulous attention to detail involved in any quantitative procedure. The remarkable consistency of replicate analyses on the same individual even when determinations of the quantity of residual air in the lungs are not made at the time of the underwater weighing is obtained in part by the necessity for agreement between the measurements of vital capacity by water displacement and by spirometry. Taking care to standardize the physiological variables or specify the parameters which affect homogeneity of composition of the *LBM*, as age, composition of diet, fluid balance, ambient temperature, state of training, time of day, alimentary gas, and residual air in the lungs, then in the same individual it is possible to obtain replicate analyses within a range for all determinations of 0.004 specific gravity units, or expressed as standard deviation, ±.0007.

In a given healthy adult male it is possible to judge the validity and accuracy of body fat values computed from specific gravity by the correspondence between weights of the *LBM* (*W–F*) over varying intervals of time, especially when the gross weight is subject to large variation. In one such individual followed over a period of 15 years during which he was subjected to the manipulations of various technical personnel and during which gross weights varied as much as 15 kg., the values for lean body weight were recorded as 70.5, 73.8, 70.7, and 70.3 kg. When the helium gas dilution technique (*21*) was used to measure body volume instead of water displacement in this subject, it was possible to compute from values of body density and body water (tritium space) substituted in Siri's formula, that the *LBW* was 71.3 kg. Applying a functional test for the estimation of *LBW*, it was found that from the subject's basal metabolism, the *LBW* could be computed as 69.8 kg. (*3*). Total body water determinations on this subject, in one case using antipyrine, in the other (3 years later) using tritum, were 49.6 and 49.9 liters, respectively (table 3).

Additional comments on the distinction between F (ether extract) and storage material (F+s) gained or lost by the adult body as a result of dietary alterations. Equations (4), (5), and (6), enable one to calculate essentially the percentage of pure fat in the body from specific gravity values. In the initial experiments, however, it was found that the density (specific gravity) of the tissue lost by an individual on a gradual weight reducing diet, was about 0.93–0.94. Subsequently (*5*), it was found that tissue gained as a result of hyperalimentation, has a density also of the same order of magnitude, but somewhat greater range (table 4), i. e., of adipose tissue. This storage tissue (the *s* component) as previously pointed out, appears to be essentially of the same composition as the bone-free (specific gravity 1.060) *LBM*. The problem would be solved if $F+s$ were simply newly formed adipose tissue (weight gain) or consumed adipose tissue (weight loss). Again, a great deal more data are required but the results of some representative experiments may be cited showing the constancy of hydration of the fat-free body irrespective of the amount of fat initially present in the body. We exclude, of course, hydration changes during rapid fat gain or loss. For example, in normal and obese mice the percentage of water in the fat-free tissue mass was not significantly different (*24*) (table 5). In cattle which varied in fat content from 15 to 45 percent, the inverse correlation between fat (ether extract) and water content was 0.99 (*11*) (table 6). In the investigations of Osserman *et al.* (*18*) the percentages of fat calculated from antipyrine space,

$$\frac{(71.8-\text{percent body water})}{71.8}$$

$$\text{Percent F}=100$$

and from specific gravity using the Rathbun-Pace equation (4), i. e., D_F, 0.918, D_{LBM}, 1.100, were not significantly different in the range of specific gravity 1.100 to 1.022, or 0.3 to 40.1 percent fat.

In the experiments of Pace and Rathbun, there was a somewhat higher percentage of water (73.04) in the fat-free tissue of ten fat guinea pigs (25.1 percent *F*) compared with 70.09 percent water in fat-free tissue of ten lean guinea pigs (4.8 percent *F*). It is tenta-

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TABLE 3

SPECIFIC GRAVITY VALUES AND LEAN BODY WEIGHT ON ONE SUBJECT OVER A PERIOD OF 14 YEARS

	Expert diving unit 1939		NMRI 1949	Expert Diving unit 1953	Donner Laboratory 1953
Specific gravity [1]	1. 056	1. 071	1.054	1. 0442	1.0448 (density).
W	92	83. 2	91.6	95. 5	98.2.
Percent "Fat" [2]	22	14. 5	23 [2]	[2] 27. 9	27.4.[3]
W "Fat"	20. 2	12. 1	21.1	26. 6	26.9.
LBW	71. 8	71. 1	70.5	68. 9	71.3.
Total water			49.9L (antipyrine)		49.6L (tritium).
Percent H_2O			70.8		69.6.

[1] Water 31°-32° C.

[2] $\Delta F = 10\,\frac{(1.100\text{-sp. gr.})}{2} \approx \frac{5.776}{\text{sp. gr.}} - 5.251 \left\{ \begin{array}{ll} \text{D "fat"} & .924 \\ \text{Sp. gr. LBM} & 1.100 \end{array} \right.$

[3] Percent $F = \frac{225}{D} - 75 \left(\frac{\text{Water (L)}}{W} \right) - 150$ (SIRI) $\left\{ \begin{array}{ll} D \text{ fat} & .900 \\ D \text{ fat f. b.} & 1.100 \end{array} \right.$

LBW calculated from BMR=69.8 kg.
Subject, age 50,H71 in.

tively concluded that the "tissue lost or gained" in response to dietary alterations, i. e., the $F+s$ substance of density 0.93 to 0.94, is inversely proportional to the specific gravity or density of the body as a whole. as is the F (ether extract. D, about 0.90). Obviously more data are required to provide a conclusive answer to this important problem.

TABLE 4

Analysis of Adipose Tissue

Species	Percent fat gross weight	Water (percent)	Fat (ether extent) (percent)	"Protein" (percent)	Water fat free (percent)	Calculated density fat
Man						
(Forbes *et al.* (*10*))	19	23	71	6	79	0. 941
Mice [1]						
(Weitze (*24*))	36	6	89	5	54	. 920
Hog	1.	17	75	8	68	--------
U. S. D. A.	2.	9	87	4	69	--------
	3.	11	84	5	69	--------
	4.	11	82	7	61	. 932
	5.	9	87	4	69	--------

[1] Hereditary adiposity.

TABLE 5

Percentages of Water (Fat-Free Weight) in Fat [1] and Normal Mice. Absolute Amounts of Water, Dry Substance and Fat in Fat and Normal Mice (from Weitze (*24*))

	Percent water		Percent fat	
	Fat	Normal	Fat	Normal
Male	72. 1	72. 9	29. 5	12. 5
Female	71. 8	74. 2	42. 5	20. 0

Absolute quantities (gm.)

	Water	Dry	Fat
Normal	17. 59	6. 35	5. 28
	73.4 percent H_2O		
Fat	18. 91	7. 36	14. 74
	71.9 percent H_2O		

[1] Hereditary adiposity.

TABLE 6

Correlations Between Fat (Ether Extract), Water, and Protein in Dressed Carcass and 9th, 10th, 11th Rib Cut in Cattle

Hankins and Howe (*11*) 120 cattle percent range fat 15.2 to 44.8.	Fat from edible portion 9, 10, 11th rib —with water portion —with protein portion —with fat, dressed carcass —with water, dressed carcass	 r = −0.99 r = −0.93 r = +0.93 r = −0.93

Number of subjects	Group specific gravity	Percent fat		Percent water LBM
		From specific gravity	From AP space	
20	1.085 (1.100–1.081)	5.56	6.15	72.0
41	1.069 (1.081–1.057)	14.2	14.7	71.7
20	1.047 (1.057–1.022)	25.15	24.42	71.8

Concluding Note

About 1943 the specific gravity technique was employed by Ancel Keys at the suggestion of this writer, in the well-known human restricted diet experiments. It has been interesting to follow the subsequent experiments of Keys, Brožek, and their coworkers (*12*), which for the most part have confirmed the results reported in these notes. They have improved somewhat the initial technique, since in replicate measurements, their standard error has been reduced to ±0.0004 density units. Fidanza *et al.* (*9*) have contributed some data which suggest that the density of human fat at 37° C. is 0.9000 gr./cc. and this is in good agreement with the Handbook value and the extrapolated value for D_F (.9018) from the Rathbun-Pace equation (4). We venture to suggest that the fourth decimal place figure is perhaps not significant. Their best estimating equation for body fat is,

$$\Delta F = (5.427/D - 5.106), \quad \begin{matrix} D_F = .9000 \\ D_{\text{reference man}} = 1.0629 \end{matrix}$$

where the reference man contains 14 percent fat. For total body fat their equation (proferred with some reluctance) is,

$$\Delta F = (4.201/D - 3.813, \quad \left.\begin{matrix} D_F = .8728 \\ D_{\text{fat f.b.}} = 1.1017 \end{matrix}\right\} \text{by extrapolation}$$

substituting 1.0629 as the "true density" of the Keys-Brozek man, for D in the formula, $\Delta F = .139$.

Substituting the specific gravity value of 1.0695 which Keys and Brožek record for their reference man, in the Rathbun-Pace equation (4), we obtain a value for $\Delta F=.143$.

To express the relationship between D and the percentage of fat in the body, let us employ the initial formula (3) of Rathbun and Pace which represented the least squares hyperbola through their data on extracted fat versus specific gravity in guinea pigs. We shall substitute D for specific gravity in their formula, then

$$\text{Percent fat}=100\,\frac{(5.135-4.694)}{\text{D}}\qquad \begin{array}{l} D_{\text{fat}}=.9018 \\ D_{\text{fat-free}}=1.0939 \end{array}$$

substituting 1.0629 for D,

$$\text{Percent fat}=13.7$$

This is the equation of choice. See table 7.

TABLE 7

EQUATIONS FOR THE CALCULATION OF BODY FAT

From specific gravity Rathbun-Pace (*20*)			From density Keys-Brožek (*12*)
$F=\frac{5.548}{\text{sp. gr.}}-5.044$ D_F 0.918 $D_{\text{fat-free body}}$ 1.100			$F=\frac{4.201}{D}-3.813$ D_F .8728 $D_{\text{fat-free body}}$ 1.1095
Percent fat = 14.3	*sp. gr.*	D	
	1.0695[1]	1.0629[1]	Percent fat = 13.9
Percent fat = 22.0	1.050	1.045	Percent fat = 19.7
From density Rathbun-Pace[2] $F=\frac{5.135}{D}-4.694$ D_F 0.9018 $D_{\text{fat-free body}}$ 1.0939			From density Keys-Brožek $F=\frac{5.427}{D}-5.106$ $D_F=0.9000$ $D_{\text{reference man}}=1.0629$
$F=13.7$		D	
		1.0629[1]	(14 percent fat)
$F=22.0$		1.045	$F=8.7\div(14)=22.7$

[1] The Keys-Brožek *reference* man, mean value obtained on 25 men.

[2] Equation of best fit for ether extracted fat and specific gravity in guinea pigs. Density (D) has been substituted for specific gravity 23°/23° C. in the original equation.

Progress is expected to be made by extending the painstaking data presented in this report to include experimental values showing the normal variation of the components of the lean body mass or fat-free body, and to clarify the nature of $F+s$. Is it in fact adipose tissue or is the s material widely distributed throughout the body as "growth substance" or storage "water and protein." We grant as do most physiologists that abnormal hydration, starvation, etc., will alter the homeostatic density of the *LBM*, and also that schizophrenic patients do not make good subjects. We are in agreement with Keys and Brožek on these points.

II. *In Vivo* Estimation of Body Fat From Measurements of Gaseous Desorption or Absorption

Since it is possible to introduce aqueous diffusible substances into the body which permit the measurement of total body water, so likewise, one may administer substances with a high (oil/water) partition coefficient. Since substances characterized by high lipid affinity are frequently toxic, e. g., CCl_4, or have other undesirable properties, e. g., dyes, one is limited to the use of such substances in their lowest concentration compatible with maximal diffusibility, i. e., in gaseous form. The realization of this objective was evident recently in the remarkably precise experiments embodying the use of cyclopropane by Lesser, Blumberg, and Steele (*14*) in their measurements of the fat content of rats. It may be of interest, by way of introduction, to review experiments conducted two decades ago in which the objective was to estimate the fat (and water) content of the body from measurements of the dissolved nitrogen in the tissues of dogs and men (*1*). Such measurements can be very precise if care is taken to control errors arising from cutaneous gas diffusion and from the initial nitrogen in the lungs during the preparatory "lung rinsing" period but they are tedious.

Manner of absorption or elimination by the body of an inert gas (*table 1*). All of the molecular nitrogen appears to be dissolved in "fluids" and fat in the ratio of about 1 to 5 or 6 except for a small quantity dissolved in hemoglobin. This gaseous nitrogen can be removed from the tissues and measured if the body is placed in an oxygen atmosphere. Dogs anesthetized by the injection of a suitable drug serve well for such experiments. If the first 7 minutes of an experiment be utilized to rinse the lungs and apparatus free of nitrogen, then during a subsequent period of 3 to 4 hours, the dog's body becomes nitrogen free. The fact that dog "G" was fat and dog "A" lean (table 1) is reflected in the nitrogen values per kg. of body weight which varied from about 11 (very lean dog) to 19 cc. (fat dog). Comparable values have been obtained in man.

Gaseous nitrogen in relation to the fluid and fat content of the body. The following values were obtained on dog *D* by chemical analysis, body weight, 12.234 kg.; fat (CCl_4 ext.), 1.889 kg.; dry solids, 3.117 kg.; and water (by difference), 7.228 kg. If these weights are multiplied by the respective solubility coefficients of nitrogen in body fat and fluids, corrected for the ambient equilibration pressure of nitrogen in the lungs (570 mm Hg.), then the nitrogen dissolved in the fat of dog "D" is 1.89 × 52 or 98 cc., and in body fluids, 7.23 × 9 or 65 cc. The total nitrogen is 163 cc. or 13 cc. per kg. of body weight.

The quantity of nitrogen recovered from dog "D" was 124 cc. The difference between 163 and 124 cc. represents the estimated quantity

TABLE 1[1]

ESTIMATION OF THE FAT CONTENT OF THE DOG'S BODY FROM MEASUREMENTS OF NITROGEN NORMALLY PRESENT IN THE TISSUES OF THE BODY

Dog (appearance)	Weight (kg.)	Experimental body nitrogen cc. (minutes)				Calculations			
		0–7	7–20	Total	Per kg.	Weight[2] fat (kg.)	Fat[3] N cc.	Weight[4] fluids	Fluid[5] N cc.
"D" lean	12. 23	39	39	163	13. 3	1. 86	96. 7	7. 49	67. 2
"A" very lean	8. 80	24	24	98	11. 1	. 92	47. 8	5. 67	51. 0
"B–2" lean	11. 30	38	38	142	12. 5	1. 54	80. 1	7. 03	63. 3
"D–1" lean	11. 90	50	50	168	14. 1	2. 02	105. 0	7. 11	64. 0
"G" fat	13. 80	65	65	258	18. 7	3. 70	192. 4	7. 27	65. 4

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[1] Adapted from Behnke, 1941–42.

[2] $F\text{-}W = LBW$. $LBW = 1.138\ W_{\text{kg}} - 0.0218$ Body N_{cc}.

[3] Fat? ×52.

[4] "Fluids" = .72 LBW.

[5] $\text{Fluids}_{\text{kg}} \times 9$.

of nitrogen eliminated during the first seven minutes or rinsing period, and is in agreement with values based upon extrapolation of the experimental curve, i. e., 30±5 percent of the body total. In practice, in order to avoid the use of extrapolated values, the quantity of nitrogen for the 7-minute period is taken as equal to the measured quantity obtained from the 7th to the 20th minute (table 1). From the total nitrogen values in the table, the quantity of fat may be computed as follows:

Let a equal weight of F, and b equal weight of fluids,
then $52\,a-9\,b=$body nitrogen cc.
If $a=$body weight $(W)-$fat-free weight (LBW), and $b=.72\,LBW$,
then $45.7\,LBW=52\,W-N_{cc}$
and $LBW=1.138\,W_{kg}-0.0218\,N_{cc}$ (1)

Measurements in man, nitrogen desorption technique (*table 2*). If a human subject is placed in an enlarged oxygen recirculating system similar to that devised for the dog, the gaseous nitrogen can also be removed from the tissues. The following data (*2*) relate to measurements of nitrogen eliminated from the body of a deep sea diver over a period of 17 hours. The specific gravity of the body as a whole was also determined on this subject.

TABLE 2

Estimation of Body Fat from the Dissolved Nitrogen in Body Tissues
(deep sea diver)

Total body nitrogen (exp.)	1076 cc.
Body weight	70 kg.
Lean body weight[1]	56.2 kg.
Fat	13.8 kg. × 52 = 717.7 cc. Fat N_2
Fluids (0.72 LBW)	40.5 kg. × 9 = 364.5 cc. Fluid N_2
	1082.2 cc.
Percent fat (body wt.) from $N_2=19.7$	
Sp. gr. whole body	1.060
Percent fat from sp. gr	20.0

[1] $LBW=1.138\,W-0.0218$ total body N_2 cc.

Computing body fat from a single gas requires an approximation of the percentage of body fluids in the LBM. If the partition coefficient is high, e. g., cyclopropane, there is only a small error involved. However, by means of the differential solubility of two inert gases in body tissues and a knowledge of their solubility coefficients, this source of variation can be eliminated.

Measurement of total body fat by absorption of cyclopropane (*table 3*). Lesser, Blumberg, and Steele (*14*) have developed with precision (for the rat) the most direct method of measuring body fat *in vivo* in terms of quantity uptake of a gas with high affinity for lipids and characterized by relatively low toxicity. Compared with nitrogen (about 5 to 1), the oil/water solubility ratio of cyclopropane is more than 52 to 1. Although this ratio in the body is reduced to about 26 to 1 due to the greater uptake of cyclopropane in protein solutions compared with water, it was found, nevertheless, that about 90 percent of the absorbed cyclopropane was contained in body fat. Wide variation in estimates of the aqueous solvent fraction, as previously pointed out, have but little influence on the accuracy of the results. The gas *absorption* technique awaits application in man. When it becomes available, possibly in connection with tracer substances, we shall have an unequivocal standard for evaluating all *in vivo* techniques aimed at the measurement of body fat. This is evident from the data in the table of Lesser *et al.*

TABLE 3

COMPARISON OF BODY FAT VALUES IN LIVING RATS AS OBTAINED BY CYCLOPROPANE ABSORPTION AND BY ETHER EXTRACTION METHODS

Rat number	Weight	By ether extraction gm.	Body fat by cyclopropane gm.	Differential gm.	Proportion of fat of body weight (percent)		
					Ether extraction	Cyclopropane	Differential
1	247	43.7	43.6	0.1	17.7	17.7	0.0
2	373	70.4	76.3	5.9	18.9	20.4	1.5
3	373	46.0	45.2	.8	12.3	12.1	.2
4	295	44.3	46.5	2.2	15.0	15.8	.8
5	307	49.6	49.1	.5	16.2	16.0	.2
6	245	28.0	26.5	1.5	11.4	10.8	.6
7	332	38.3	35.3	3.0	11.5	10.6	.9
8	270	25.0	22.1	2.9	9.3	8.2	1.1
9	236	26.8	28.4	1.6	11.3	12.0	.7
10	355	28.8	31.4	2.6	8.1	8.8	.7
mean	303	40.1	40.4	±2.1	13.2	13.2	±.7

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M. F. Morales (Naval Medical Research Institute):

There is probably no one here whose research is more distant from this subject than is mine, and ordinarily I would feel very silly indeed in addressing you this way, but en route to this meeting I read a review which I take to represent current thinking on this problem, and this has reassured me that a few platitudes are not entirely out of order.

I would like to go over some of this well-trodden ground, but with my own symbolism, merely because it is more familiar. The fundamental ideas in this work are Captain Behnke's, as you all know; my participation in this development consisted in the mere translation of these ideas into formal language, and, as analysis goes, this was no trick at all, but consisted essentially in writing down the definition of density.

Density, D, is mass/volume. If one considers the human body to consist of a lean mass, M_1, and an adipose mass, M_a, then the numerator of our ratio is $M_1 + M_a$. If D_1 is the density of the lean mass, then its volume is M_1/D_1; if D_a is the density of adipose tissue, then M_a/D_a is its volume. If the lean and adipose "components" do not intermix, i. e., *if their volumes are additive*, then the denominator of our ratio is $(M_1/D_1) + (M_a/D_a)$. Rearrangement of this formula leads to the symmetrical expression,

$$\frac{M_a}{M_a+M_1}=\frac{(1/D)-(1/D_1)}{(1/D_a)-(1/D_1)} \tag{1}$$

For the stated problem, equation (1) is exact, i. e., it is the correct relation between the weight fraction of adipose tissue in a given *individual* and the three densities measured in the *same individual*. Behnke suggested, however, that among various individuals of a population (chosen with some restrictions, of course; for instance it clearly would not include edematous patients or circus freaks) or in the same adult at various times both D_1 and D_a were sensibly constant. Acceptance of this postulate converts (1) into the form,

$$\text{(2)} \qquad \text{Adipose Fraction} = \frac{\text{Constant } A}{D} - \text{Constant } B.$$

Equation (2) now applies to the *population*. Constants A and B are ultimately obtained by statistical analysis of paired measurements of Adipose fractions and D's on a sample population, or from dissecting tissues measuring D_1 and D_a directly.

Formula (1) was adopted and successfully used by Pace and Rathbun, who went on to evaluate A and B by statistical analysis. I was flattered to learn that Dr. Brožek has also extensively adopted our formula. On the other hand we, and later, in a far more conclusive way, Pitts, measured D_a and the densities of the subcomponents (bone, muscle, etc.) of D_1 by direct dissection: incidentally, we found that the limiting D (adipose fraction, zero) in a human population coincided remarkably well with D_1 from direct dissection. These various observations unquestionably endorse equation (2), and with it Behnke's postulate.

Recently there has been much ado about the value to be used for D_a. In my opinion this depends on the interesting cytological problem of whether adult adipose tissue grows by what is effectively proliferation or whether it grows by accretion of fat as a chemical substance (there seems to be some evidence that both processes may occur). If it grows by proliferation one must use the value obtained by densitometry of adipose tissue (in our guinea pig work we found this to be between 0.92 and 0.93); if by accretion of fat, then one must use the value for chemical fat (closer to 0.90). *But in either case equations (1) or (2) apply;* this is one of the necessary platitudes I had in mind. This same question of how adipose tissue grows or regresses is at the root of another current controversy, viz., the relation between densitometric estimates of adipose mass and estimates based on the measurement of total body water (e. g., antipyrine dilution) or total body fat (cyclopropane dilution). If adipose tissue grows by accretion of fat as a chemical entity, then the densitometric and dilution methods are easy to reconcile using the present analyses; if growth is by cell proliferation or cell recruitment, however, allowance has to be made for the water in the adipose tissue, and this calls for slight modifications of the analysis—modifications which have been slow in coming.

Not all recent critical comment seems to have dealt with real issues; a complete enumeration of bogus issues would be confusing and unproductive, but two should be pointed out before they are dignified by mere repetition. It is not true, for instance, that agreement between densitometric and dilution methods is logically spurious (in the sense that both methods depend on the same assumption). It is also not correct to apply equations (1) or (2) to systems of chemical components (e. g., water and solids) if the components interdissolve to any extent, for in the derivation of these equations, as you will recall, is the assumption that the volumes of the components are additive.

On the searching examination to which Behnke's thesis has been recently subjected, I have mixed reactions. It has been good, for instance, that conceptual errors such as the confusion of density with specific gravity have been cleared up. It is also good that Dr. Brožek and others have become interested in more accurate measurements of the necessary parameters—they might in fact do well to examine nonuniform concentration errors in the dilution methods too.

But it would be a pity indeed if preoccupation with accuracy distracted or detracted from what is unquestionably a good first approximation, that is, if one lost sight of the forest for the "Keys".

BASS:

For our purposes, are we really interested in the right parameter that is between lean fat mass and fat? Should we not attempt to dissect the body in terms of our other components? For instance, it always seems to me the concept of the fat-free mass which was expressed by Captain Behnke with the limitations has been extrapolated by people who have been using the term freely, and treating it as a single physiologic parameter which acts as a single parameter. Of course, that is not so. Obviously, we have two major media, intra- and extra-cellular, which our data indicates seems to be responsive to at least, in part, independent physiologic mechanisms in individuals. On BMR and our 54 men, it indicates our intra- and extra-cellular foods are not reciprocally related, but can be different in different individuals without one being affected by the other, and I think we should always be careful in thinking of the fat-free mass, in thinking of it just as a means of skimming off from the total body weight so we can get the fat and nothing else. In other words, physiologically, it may not be too meaningful.

BEHNKE:

It might be better to think of constancy as structure. We mean really homeostatic. A tendency to come to equilibrium, bring up the body temperature at a certain time of day in healthy individuals, is about the same magnitude, and so is the composition of the body. It is obvious that excess fat is the chief variable affecting the homeostatic constancy. One of these compartments or the extracellular space was examined first, then total body water. We would like to know the mineral content and then protein content. It may be possible in time to have all of these gross entities and then add them up and find out what their relationship is.

There is one thing that surprises me. I don't know how you can relate the careful measurement you made on extracellular fluid with your study—what is your standard of estimating what the fat-free body is?

BASS:

We use skinfolds and we don't think we demonstrated it wasn't a true balance.

BEHNKE:

There is something very interesting about the extracellular fluid volume; from your work it appears it bears a rather constant relationship to gross weight rather than lean weight, but it is difficult for me to see how it would be a better reference standard with reference to the fat-free body than total body weight, because the percentage of the total body weight is largely intracellular water, which varies much less than the extracellular water.

On general grounds, it would seem that the total body water would have a higher correlation with the fat-free body than the extracellular fluid volume, which contains two things that vary a great deal—the water between the cell spaces and the blood volume.

BASS:

We have parceled out the blood volume or the plasma volume in our studies, but we did not find there was a better correlation with total body weight, but rather with fat-free mass, as determined by skinfolds.

BEHNKE:

Has it been the experience of the analytical chemist and nutritionist when fat is laid down, that there is excess water that accompanies the laying down of this fat, perhaps in the extracellular fluid space?

BASS:

Dr. Brožek has attempted to answer that question in his review article. I think your claim is that there are extracellular fluids?

MODERATOR GROSSMAN:

Perhaps a partial answer to that might come from the analysis of carcasses of animals with the experimental obesity. I have looked at some of that data and made calculations from them. For example, in the obesity, mucus-induced obesity, if you take the carcass analysis for the control group and the carcass analysis for the obesity group and subtract them and calculate the percentage of the obesity tissue, it comes out very close to the analysis given by Keys and Brožek, with about close to 70 percent fat and about 15 percent water. I don't know of any other direct evidence of that kind, because, in other instances, you are dealing with growth in addition to the laying down of the fat.

BEHNKE:

There is obesity tissue, and that is the whole thing that the histologists and anatomists call adipose tissue. It has about the same density.

BROŽEK:

I have only one other comment, and that is on the work done at Lawrence. There are two problems. One, as far as the relative value of the lean body mass predicted from the skinfolds, and the correlation of fluids is the other method. I can cite you your numbers, and correlation is not dependent on the main value. All they did is show the association between two series of numbers.

My interpretation would be that the particular type of calipers that has been used in the study reported by Bass, was one in which the tension was smaller than we had and was different from the tension, and the equation that we have published in the partition of nutrition gives absolutely reasonable values only if the skinfolds are measured at the same tension at which the origin would obtain.

MODERATOR GROSSMAN:

I would like to take the prerogative of the chairman and make one brief, final statement. In spite of all of the apparent controversy among the experts, there is a very real usefulness for these methods. I think that everyone here will agree that so far as practical evaluation of nutritional status is concerned, that the skin caliper method is now in a state of development where it can give us full and useful results.

(The meeting adjourned at five o'clock.)

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